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the third year. A: 12 per cent of the main-stem growth of 13 or 15 + N, 84 per cent of 10 – N trees died back within the year of injection. No wilt or die-back developed on any trees the second year after injection. B: 0.4 per cent of the branch growth of the 6 + N, 35 per cent of the 10 – N trees died back within the year of injection. Similar trees were cut back and grown with daily applications of complete nutrient solution, or the same minus nitrogen. They were injected the second season. Wilt developed very quickly on 51 of 54 + N trees. Within 17 days 88 per cent of their leaves were lost, 41 days after injection 93 per cent of their main-stem growth died back. Wilt developed extremely slowly on 40 – N trees. Six displayed wilt within 17 days, 34 within 76 days. By season's close 73 per cent of their main-stem growth died back. No wilt or die-back developed in control trees of any group.

The Relation of Sap Flow, Vessel Length, Spore Distribution to Development of Dutch Elm Disease in American Elm. W. M. BANFIELD. *Ceratostomella ulmi* spores, beer yeasts, India ink, and iron colloids were injected into 135 American elms during the growing season. One hour after injection *C. ulmi* was recovered up to 50 feet in five 60-foot trees and up to 62 feet in one 65-foot tree. The colloids used, $Fe_4(Fe(CN)_6)_3$, $Fe(Fe(CN)_6)_2$, FeOCl, could not be detected more than 10 feet above injection points in comparable trials. Beer yeasts were recovered 25 feet above injection points. India ink was observed to descend 36 feet below injection points in 6 minutes. *C. ulmi* was recovered 40 feet below injection points 1 hour after injection in trials on 2 trees injected 41 feet above the ground. The locus of major spore distribution in all cases was the ring of large spring vessels of the current season's growth sheath. All earlier rings were found closed by tyloses in the crown and top half of the bole of 75 trees examined. Root vessels were tylose-free. Maximum spore distribution in summer wood vessels was 15 inches. Spore distribution prior to leaf expansion in the spring was limited to a few inches. No spore distribution in new vessels occurred until their partition walls had disappeared. Ten days later first symptoms of disease appeared in naturally infected trees.

Persimmon Wilt. R. KENT BEATTIE and BOWEN S. CRANDALL. A hitherto unrecognized rapid-killing wilt of the native American persimmon has made its appearance in Tennessee, southeast of Nashville. The known area of infection, based on rather limited scouting, is small. It consists of local spots in a triangular area between Lebanon, Bradyville, and Columbia, Tennessee. Nearly all the infections are 1 to 2 years old and only 2 are 4 to 7 years old. Although the disease has not yet been associated with any one known abroad, it behaves like an introduced disease. Infected trees are conspicuous in early summer because of their wilted and discolored leaves. Brownish-black streaks are formed in the recent annual rings. A species of Cephalosporium, isolated, and grown in culture, has produced the disease on inoculation into American persimmon, and has been reisolated. Spores are produced in openings and cracks in dead trees and especially under loose bark, where they may be collected by spoonfuls. They are probably windborne. Other species of persimmon have not yet been tested for susceptibility. No remedy is known.

Illustrations of South American Elsinoe and Sphaceloma Diseases Known up to January, 1936. A. A. BITANCOURT and ANNA E. JENKINS. A recent general article on the diseases of plants caused by *Elsinoe* and *Sphaceloma* contained a section relative to the 13 members of the group found in South America. This section was accompanied by a synoptic table giving available literature citations or other records relative to this special group. Representative illustrations were not included in the published paper, that is, with one exception. Illustrations representative of all 13 diseases are, therefore, assembled here for convenience of reference in connection with the paper cited, or with subsequent reports of the 13 fungi, or diseases, or with the considerably larger number subsequently discovered in South America, particularly in Brazil. Certain additional notations are made relative to diseases known up to January, 1936.

Mechanical Transmission and Properties of the Potato Yellow Dwarf Virus. L. M. BLACK. Leaf-hopper-borne viruses have been transmitted mechanically only with difficulty or not at all. The potato yellow-dwarf virus, readily transmitted by the clover leaf hopper, *Aceratagallia sanguinolenta,* could not be transferred mechanically from clover to clover, although clover is susceptible. Mechanical transmission from potato to potato is difficult, the best method tested giving only 50 per cent infection. In *Nicotiana rustica,* however, mechanical transmission is readily obtained. Rubbing nondiluted juice from diseased *N. rustica* plants over leaves dusted with carborundum may result in the production of as many as 3000 yellow primary lesions per leaf. The primary lesions were employed in making quantitative determinations in studies on the properties of the virus. The virus passes through a Berkefeld W filter, has a dilution end-point of 1×10^{-3},

resists aging *in vitro* for 3 to 4 hours, and is inactivated by heating for 10 minutes at about 50° C. Attempts to recover the virus from dried leaves have failed.

A Sugar-beet Root Rot Caused by Aphanomyces cochlioides. W. F. BUCHHOLTZ and C. H. MEREDITH. In August, 1937, 61 per cent of a random sample of sugar-beet tap roots from Kanawha field were or had been rotted. Brownish discoloration and a water soaked, slightly softened consistency characterized the lesions. Many rotted tap roots had become desiccated and shrunken until only a fibrous tassel-like structure remained. Such plants experienced increasingly severe wilting and eventual death, although the latter was sometimes delayed by an excessive number of short slender side roots. Isolations on August 21 and 23 yielded 2 and 6 cultures of *Aphanomyces,* and on August 31 and September 21 *Aphanomyces* was recovered in 11 of 12 and 23 of 24 trials. Bacterial contaminants were common. Two plug-inoculation experiments in the field yielded 100 and 91 per cent rotted beets. A similar experiment with harvested beets yielded 91 per cent. Symptoms were different from those produced by *Phytophthora drechsleri* inoculations. *Aphanomyces* was reisolated in each experiment. Checks were not rotted. In typical cultures, zoospores emerged as delimited protoplasts from unbranched hyphae and were motile in 12 hours. Oogonia with branched enveloping antheridia were present in 2-day-old cultures. Modal size was 24 μ, range 24 μ to 29 μ. The cultures studied have been assigned to the species, *Aphanomyces cochlioides.*

Pythium de baryanum and Other Pythium Species Cause Alfalfa Seedling Damping Off. W. F. BUCHHOLTZ and CLIFFORD H. MEREDITH. Cultures of 5 species of Pythium, *P. de baryanum, P. splendens, P. pulchrum, P. rostratum* and *P. ultimum,* have been isolated from field-grown, damped-off alfalfa seedlings and shown to be pathogenic under greenhouse and laboratory conditions. In 50 isolation trials with alfalfa seedlings from early 1937 plantings, 43 yielded *P. de baryanum.* In later trials, 53 of 68 isolates were *P. de baryanum.* This pathogen, as in 1935 and 1936, was the one most commonly isolated. April or early may, 1937, plantings of alfalfa and other legumes and sugar beets in 8 fields in southern, central, and northern Iowa yielded uniformly good emergence and survival stands much superior to those from later May plantings. In general, late-planted stands of red clover and sweet clover were superior and late-planted stands of sugar beets were relatively inferior to late-planted alfalfa stands.

Control of Internal Cork of Apple with Boron. A. B. BURRELL. In a 27-year Fameuse orchard, boron treatments on alternate trees reduced internal cork as follows:

(a) Injection of dry boric acid, from 36.45 per cent of the apples (average of 27 check trees) to 0.22 per cent (average of 27 treated);

(b) 1-pound borax on soil, from 13.40 per cent (average 15 checks) to 0.06 per cent (average 16 treated);

(c) ½-pound borax on soil, from 24.99 per cent (average 25 checks) to 0.10 per cent (average 24 treated); all based on slicing 200 fruits per tree.

Limited data on McIntosh and Cortland in 3 other orchards are corroborative. Each injection killed a small strip of bark, but foliage appeared unharmed. No injury is apparent yet from spring (1937) soil application of borax to about 15,000 trees. Thirty-six young trees received liberal soil applications of boron August 13, 1936. In 1937, on 6 of these certain leaves each developed a sizeable white area at the proximal end extending along midrib and main veins. An identical pattern resulted from dipping growing shoots in boron solutions. Two pounds of borax per 100 gallons of common spray mixtures caused mild marginal scorching on immature leaves unless either lime or lime sulphur was present; mature leaves appeared uninjured. Boron seemed ineffective against bitter pit.

Fungi Associated with Oak Diseases. J. C. CARTER. A survey of little-studied oak diseases, initiated in Illinois in 1934, is still in progress. Branch and twig cankers, dieback, twig blight, deep wood infections, and staghead have received special attention on *Quercus alba, Q. bicolor, Q. borealis maxima, Q. ellipsoidalis, Q. imbricaria, Q. macrocarpa, Q. marilandica, Q. muhlenbergii, Q. palustris, Q. stellata,* and *Q. velutina.* Of 137 cankers examined, 71 (group one) bore fruiting bodies of fungi obviously associated as causes of the cankers, while 66 (group two) gave no outward evidence of cause. Microscopic examination of group one revealed species of *Bulgaria, Coryneum, Cytospora, Diatrype, Phoma, Phomachora, Rhodosticta, Sphaeropsis,* and *Ustulina;* and cultures gave species of *Alternaria, Aspergillus, Chaetomium, Coryneum, Cytospora, Fusarium, Penicillium, Phoma, Phomachora, Phomopsis, Rhodosticta,* and *Ustulina.* Cultures of group two gave species of *Alternaria, Coryneum, Fusarium, Penicillium, Phoma, Phomachora, Phomopsis,* and *Ustullina.* From 51 cases of dieback, twig blight, deep wood infections, and staghead, cultures gave species of *Alternaria, Armillaria, Coryneum, Cytospora, Fusarium, Penicillium, Phoma, Phomachora, Phomopsis,* and *Ustulina.* From wood discolorations extending

backward into otherwise healthy wood, only *Phomachora* and *Cytospora* were secured in cultures. The fungus most frequently found associated with staghead and sudden dying was *Armillaria mellea.*

Basicop as a Cherry Spray. DONALD CATION and COLIN W. ROBERTSON.

Cherry-spraying Experiments in Michigan, 1937. DONALD CATION and E. J. RASMUSSEN. Experiments conducted at Hart and East Lansing, Michigan, comparing certain spray materials for the control of cherry leaf spot (*Coccomyces hiemalis*) included lime sulphur, Bordeaux, Basicop, Bordow, copper oxychloride (3 brands), Copper-Hydro 40, 66A, 66B, Z-O, Oxo Bordeaux, copper phosphate, Coposil, and basic copper chloride. A preblossom infection together with well-distributed rain periods were favorable for heavy defoliation due to leaf spot. Lime-sulphur generally failed to control the disease, while most of the copper materials gave adequate protection. These materials were evaluated on the basis of control, injury to the foliage, and resulting size of fruit.

The Occurrence of Lysis in Certain Crosses of Ustilago zeae. ST. JOHN P. CHILTON. Chlamydospores resulting from crosses between certain haploid lines of *Ustilago zeae* germinated abnormally and the promycelia disintegrated. Not all of the promycelia produced by chlamydospores from any one cross disintegrated, but normal promycelia with 4 sporidia were found very infrequently. This lysis appeared in all crosses in which the lethal factor or factors involved were present in one of the parent lines and absent in the other. These lines are the progeny of a single original cross, and the abnormality has persisted through 3 chlamydospore generations. The same original cross has produced haploid lines also, which have remained normal through five chlamydospore generations. When grown in culture, haploid lines resulting from crosses containing the lethal factor or factors were less vigorous than the lines resulting from crosses in which this abnormality did not appear. Abnormal meiotic divisions may occur, as solopathogenic lines were obtained, which, with one exception, arose from crosses involving the lethal factor or factors.

Root Rot of Pines Caused by Armillaria mellea. CLYDE M. CHRISTENSEN. Several hundred jack pine, *Pinus banksiana,* Norway pine, *P. resinosa,* and northern white pine, *P. strobus,* growing in natural forests, were selected at random, dug up, and the roots examined for infection by *A. mellea.* The infection varied from 0.0 per cent on jack pine 5–10 years old to 100 per cent on Norway pine 250–300 years old. Infection was more common in dense stands than in open stands. Suppressed, weakened, or injured trees in dense stands did not appear to be more susceptible to infection than dominant, vigorous, uninjured trees in the same stands. The damage appeared to be greatest in stands under 80 years and over 200 years of age. Rhizomorphs, apparently of *A. mellea,* were present on the roots of practically every woody plant that was dug up, indicating that the fungus probably is generally distributed in the forest. Numerous cultural strains of the fungus were isolated, but none of them was found to attack seedlings of pine and spruce grown in pure culture. The dying of old, over-mature Norway and northern white pines was attributed chiefly to root rot and drouth.

Mosaic Resistance in Nicotiana tabacum. E. E. CLAYTON, H. H. SMITH, and H. H. FOSTER. Seed collections from Mexico, Central America, and South America were tested for resistance to common tobacco mosaic. Thirty-six out of 897 proved resistant, and all resistant lots came from Colombia, South America. Included, were a number of varieties quite distinct from Ambalema. Resistant collections fell into 2 groups: the highly resistant showing none to abundant localized light green spots, and the moderately resistant showing mild systemic mottling. The resistance reaction of the first group, which includes Ambalema, was conditioned by 2 major recessive factors, with indications that this resistance may be affected to some degree by modifying factors.

Fusarium Wilt and Stunt of Spinach in Virginia. HAROLD T. COOK and T. J. NUGENT. A Fusarium wilt of spinach, observed in Virginia for the last 7 years, is becoming increasingly important. It now causes an annual loss of about 3 per cent of the crop, and the loss in some fields is as high as 90 per cent. It is most destructive to spinach planted in late August and in September and to spinach grown for seed. Wilting does not occur until the plants are about 3 weeks old and may not take place until even later. Symptoms of the disease in the young plants are dull green, limp foliage followed by wilting, destruction of the fibrous roots, black lesions on the tap root, and a brown discoloration of the stele. Symptoms in the older plants are stunting, yellowing and wilting of the outer leaves, a reduced root system, and brown discoloration of the stele. Cool weather retards the disease, and plants in which infection has not progressed too far often form new roots and make a good growth late in the fall. The fungus that causes stunt and wilt does not decay the seeds or cause damping off; seed treatment is ineffective as a control measure.

Experiments in Crossing Varieties of Puccinia graminis. RALPH U. COTTER and MOSES N. LEVINE. Pycnial nectar of *Puccinia graminis agrostidis* was mixed in various combinations with that of *P. graminis tritici, P. graminis secalis, P. graminis poae,* and *P. graminis avenae,* respectively. While aecia resulted from less than 35 per cent of 382 crosses attempted, some were produced from every combination, although no uredial cultures were obtained from *agrostidis* × *avenae* or its reciprocal. Positive results include: 16 *tritici* and 2 *hordei* races from *tritici* × *agrostidis*; 5 *tritici,* 2 *hordei,* and 1 *secalis* from *agrostidis* × *tritici*; 9 *tritici,* 4 *secalis,* and 1 *avenae* from *secalis* × *agrostidis*; 4 *tritici* and 1 *secalis* from *agrostidis* × *secalis*; 3 *tritici* races from *poae* × *agrostidis.* A few *tritici* and *secalis* races isolated attacked many of their respective differential hosts. Some isolates were common races but several strains were new. The one *avenae* strain isolated was race 5, and three of the *hordei* races were new. While none of the *hordei* races could succesfully attack any other grain besides barley, they differed in parasitism on three varieties of barley. Finally, a strain was isolated from a cross of *P. graminis tritici 17* × *P. graminis agrostidis* producing fairly severe infection on Gopher oats in addition to attacking normally different varieties of barley. (Division of Cereal Crops and Diseases, Bureau of Plant Industry, and Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in cooperation with the Minnesota Agricultural Experiment Station.)

Inhibitory Substances Generated by Gibberella saubinetii. G. N. DAVIS. An inhibitor was produced in 14 days by *Gibberella saubinetii* in Czapek's liquid media in which ammonium tartrate was used as a source of nitrogen. The filtrate was removed from the flasks and added in various dilutions to hard Czapek agar. The growth of *G. saubinetii* at 15° C., on agar plates containing 50 per cent or more of the filtrate reduced to 50 per cent of that made by the check. On other plates, containing 30 per cent of the filtrate, the growth was 75 per cent. When talc was added to the filtrate (1 gm. per 100 cc.) as an adsorbent and stirred vigorously for 20 minutes, approximately 80 per cent of the inhibitor was removed from the filtrate. The inhibitor retarded *Gibberella* in infected seeds. Infected seed, soaked for 24 hours in the inhibitor, gave a 59 per cent germination at 10° C. Seed from the same lot, treated with ethyl mercury phosphate, showed 57 per cent germination, and the checks 46 per cent.

Smut in Latent Buds of Sorghum. G. N. DAVIS. When the terminal panicles of the sorghum plant are removed the latent axillary buds become active, producing new panicles. *Spacelotheca sorghi* may infect many of the growing points in the terminal shoot, as already shown by the writer for *Ustilago zeae* of maize. This is apparent from the following experiments. Four orange cane plants, naturally infected with *Spacelotheca sorghi,* planted on May 14, 1935, are actively growing in the greenhouse and continue to produce smut-infected panicles. During these 2½ years, 122 smutted panicles have developed on 4 plants. Six plants of a similar series, 1½ years old, have yielded 78 smutted panicles and 14 healthy ones. The check plants never produced a single infected panicle. Plants inoculated by the hypodermic-needle method when about 30 days old, yielded 14 smutted and 11 healthy panicles. Smut in the plants hypodermically inoculated developed only in panicles from axillary buds on inoculated culms. Noninoculated culms produced smut-free panicles.

The Black-seed Disease of Strawberry. J. B. DEMAREE and MARGUERITE S. WILCOX. An occasionally important minor disease of strawberry fruits, caused by the leaf-spot fungus, *Mycosphaerella fragariae,* has been observed in Maryland and North Carolina. The designation "black-seed" has come into general use by growers, owing to the black color of affected achenes and the epidermis of the receptacle immediately surrounding their attachment. The blackened achenes are quite conspicuous on the white unripe fruits, and on ripe fruits of light-colored varieties. Usually, only 1 or 2 spots occur on affected berries, but, occasionally, as many as 8 or 10 spots are found. It is believed that infection enters by way of the stigma, and the fungus ramifies throughout the seed structure and closely associated pulpy tissues. A fungus has been isolated from the infected pulp and achenes resembling morphologically and culturally the conidial stage of *M. fragariae.* The black-seed disease was produced in the greenhouse by inoculating young berries with cultures of the fungus isolated from the infected berry tissue, from strawberry leaf-spot lesions, and from ascospores of *M. fragariae.* Typical lesions of strawberry leaf spot also were produced by inoculating young strawberry leaves with the isolate made from the black-seed-affected berry tissues.

A Simplified Technique for Tobacco-virus Purification and Crystallization. B. M. DUGGAR and WAYNE E. MOORE.

A Survey of the Fungi Associated with Wood Decay in Apple Trees. CARL J. EIDE and CLYDE M. CHRISTENSEN. Sixty-eight apple trees, 22 years old and well cared for,

were sawed off about 3 feet above ground and again at ground level. Of these, 12 were badly decayed, 22 moderately so, 22 slightly rotted, while in 12 there was no visible sign of decay. Fruiting bodies of *Daedalia unicolor* were found on one of the badly decayed trees and *Polyporus versicolor* on another. In another orchard, neglected for over 10 years, fruiting bodies of the following fungi were found on decaying trees: *Fomes applanatus, Trametes hispida, T. malicola, Pholiota adiposa, Polyporus versicolor,* and *Thelephora* spp. Cultures were made from the fruiting bodies and from decayed wood, and it seems probable that these fungi are responsible for active decay in living trees. Several other Basidiomycetes were isolated from decaying apple wood, one of which has been identified provisionally as *Polyporus resinosus.* Fruiting bodies of the following fungi were observed on dead branches of apple trees: *Polyporus tulipiferas, P. pubescens, P. versicolor, Lenzites trabea,* and *Daedalia unicolor. Schizophyllum commune* is abundant on sun-scald cankers and other dead bark. It was isolated from dead bark but never from decayed wood.

Buckwheat as a Factor in the Root Rot of Conifers. E. J. ELIASON. A severe root rot appeared during a warm period in a block of red-pine transplants, in June, 1933, at the Saratoga Nursery of the New York State Conservation Department. It was noted that the rot was found in parts of the nursery, successively sown for three or more years with buckwheat as a cover crop. Field and laboratory studies have shown definitely the relation of the buckwheat residue to the rot. The fungus appears to be one or more species of *Fusarium.* High temperatures increase the severity of the rot. Tests of susceptibility have shown red pine to be the least resistant. Norway spruce proved to be almost immune, while white spruce and white pine are intermediate. Transplants suffer the greater loss during the first year. Infected soils also cause damping off and root rot to one year seedlings in the seedbeds. Control by means of soil treatment has met with little success. Areas on which no additional buckwheat or other organic materials have been added, still produce severe rot after 4 years. Practical control has consisted of the planting of susceptible species on noninfected areas and early planting to avoid higher soil temperatures of late spring.

Root Infection of Allium Species by Urocystis cepulae. E. L. FELIX. Mature smut lesions resembling those on the cotyledons and true leaves occurred occasionally in the field and greenhouse on primary and secondary roots of 4- to 6-week-old seedlings belonging to *Allium cepa, A. fistulosum,* and *A. porrum.* Infected areas, which varied from 1/32 to over an inch in length, ranged from brown to black, depending on the number and maturity of spores. Invasion of both primary and secondary roots seemed invariably to originate from infections in the region of the root joint, either of the sheath itself or of the meristematic "plate." The sheath normally surrounds the forming secondary roots, which stretch and ultimately rupture it, leaving a temporary collar at the base of the root or carrying a sleeve-like portion of it anywhere on the root. Smut hyphae are intercellular in the roots, as in the cotyledon. Mycelium and spores occurred infrequently in the root hairs.

The Isolation and Identification of the Pigment Present in Cultures of Actinomyces violaceus-ruber. VERNON L. FRAMPTON and C. F. TAYLOR. The pigment elaborated by *Actinomyces violaceus-ruber* has been identified as an anthocyanin, and has been isolated as the crystalline picrate. The pigment is a rhamnose glucoside, the carbohydrate groups being rhamnose and glucose. Solubility studies suggest that the sugars are separately attached to the anthocyanidin residue. The anthocyanidin remains to be identified.

Physiological Variation in Isolates of Polyporus schweinitzii. WM. D. GRAY. Weighed wood blocks of white-pine sapwood were inoculated with ten different isolates of *Polyporus schweinitzii,* obtained from various localities and various coniferous hosts. Cultures were maintained for 10 months; the blocks were then air-dried, weighed, and percentage weight losses were obtained for each series. Statistical analysis of results reveals that striking differences in wood-decaying ability exist between various isolates of this species.

Alkaloids Isolated from Plants Resistant to Phymatotrichum omnivorum and Their Influence on Growth of the Fungus. GLENN A. GREATHOUSE. Preliminary studies indicated the presence of alkaloids in crude extracts from roots of several plants resistant to *P. omnivorum.* Six alkaloids have been isolated in pure form from these extracts, and their influence on growth of the organism determined. Only the nitrogen content and the physical properties of these compounds have yet been determined. Similar extracts from roots of several susceptible plants did not yield crystalline alkaloids. The toxicity of the crude extracts on the growth of *P. omnivorum* in certain instances was greater than

the toxic effect that could be ascribed to the amount of alkaloids isolated from a given unit of extract. This indicates that the quantity of alkaloids isolated either represents only a fraction of the total present or suggests that still other entities may be responsible for a portion of the toxicity to the organism. A search is being made for other possible chemical entities that may be responsible for a portion of the resistance to the fungus. In addition to the alkaloids isolated, 27 other alkaloids from resistant and susceptible plants have been obtained through several investigators and their effect on the organism recorded.

Parasitism and Physiologic Specialization in Fomes lignosus. EARLE W. HANSON. A Liberian culture of *Fomes lignosus* caused severe infection on *Hevea brasiliensis* and *Ficus elastica* in artificially inoculated soil. Typical symptoms of the white-root disease appeared 5 weeks after inoculation, and in approximately 6 weeks the plants were dead. Checks remained healthy. The pathogen was reisolated from the roots of dying plants and found to be identical in all respects with the original culture. Histological studies showed that the fungus was abundantly present in the diseased roots. Similar investigations with a number of leguminous crops showed that navy beans, soybeans, cowpeas, and garden peas also are susceptible to *F. lignosus.* A culture of the fungus obtained from Malaya was compared with the Liberian isolate and found distinctly different in cultural characters, rate of growth, temperature and moisture requirements, and in pathogenicity. On most media the Malayan culture produces a finer, faster growing mycelium than does the Liberian isolate. It has an optimum temperature of 32° C. as compared with 27° C. for the latter. Its moisture requirements for optimum growth are greater than for the Liberian, which grows best under conditions of relatively low moisture. The Liberian isolate is much more pathogenic on legumes than is the culture obtained from Malaya. (Minn. Agr. Exp. Sta. and Firestone Plantations Company, cooperating.)

Preliminary Studies on the Cytology of Urocystis waldsteiniae. E. W. HANSON and R. E. ATKINSON. In *Urocystis waldsteiniae* the haplophase apparently has been almost eliminated. The fusion nucleus apparently undergoes meiosis in the chlamydospore, as very young promycelia are binucleate and all subsequent vegetative development is dikaryotic. Individual cells of septate promycelia, and sporidia, are almost invariably binucleate. The mycelium in the host is usually multinucleate, at first, but becomes binucleate with the formation of septa. Even in nonseptate hyphae, the nuclei are commonly associated in pairs. Chlamydospores are binucleate until almost fully developed, when, following fusion, they become uninucleate. Chlamydospores of *U. waldsteiniae* require no rest period. They germinate readily in water within 48 hours, normally producing a single promycelium, but occasionally more. Sporidia, germ tubes, or both, usually 2 in number, are produced by the young promycelium, either laterally or 1 laterally and 1 terminally. Sporidia are very large and readily abstricted. They have never been observed to bud, but germinate from one or both ends, or occasionally from the side. Promycelia, likewise, may become detached from the spore and behave like sporidia.

Blue Rot of Boxwood. J. G. HARRAR. Isolations from over 300 diseased boxwood plants from various parts of Virginia indicate that *Verticillium* species are invariably associated with the so-called blue rot of *Buxus.* On the basis of morphological and cultural characters, 9 apparently distinct strains of *Verticillium* have been isolated from the diseased plants. Infection of healthy boxwoods has been obtained with 3 of these strains. From experimental evidence, it seems probable that the organisms involved are only weakly parasitic and that predisposition is essential to infection.

Phoma (Phyllosticta) antirrhini in Virginia. J. G. HARRAR and L. I. MILLER. The Phoma disease of snapdragons has been wide-spread in Virginia during the past three years. The causal organism has been identified as a species of *Phoma,* probably identical with the organism previously reported as *Phoma* and also as *Phyllostica antirrhini.* Moreover, since other members of the Scrophulariaceae have been successfully infected with this fungus, it may possibly be identical with other species reported on these host plants. As recorded earlier, *P. antirrhini* causes damping off of young plants and produces stem cankers and leaf spots on older hosts. In addition, it has been found to produce severe seed-pod infection. In the field, maximum injury occurs on older plants. It has been demonstrated that the disease is both soil- and seed-borne, and experimental results indicate that seed selection, seed treatment, and sprays offer practical control measures.

Microchemical Studies of Potato Tubers Affected with "Blue Stem." L. M. HILL and C. R. ORTON. A technical description of this disease appeared in Jour. Agr. Res. 55: 153–157. 1937. Tests for cellulose showed this compound to be absent or frag-

mentary in the cell walls of the necrotic areas of parenchyma and phloem, while tests for cellulose and lignin were positive in the walls of necrotic xylem. Pectin was present in healthy but absent in diseased tissues. Suberin was detected in the necrotic parenchyma, phloem, and xylem. Protein seemed to be absent within diseased regions. Fat was found uniformly distributed throughout diseased and healthy tubers, although the yellow precipitate, associated with necrotic regions, gave a negative fat test. Glucose was detected in healthy and diseased tubers, but with greater concentration in the necrotic regions. Sucrose was found in very small quantities in both healthy and diseased tubers; the same applied equally well to potassium, phosphates, magnesium, calcium, and sulphates. Iron was concentrated around diseased areas. No nitrates were found in the necrotic areas and adjoining cells. Oxidase was concentrated in a zone surrounding the necrotic regions.

Pythium graminicolum on Barley in Iowa. WEN-CHUN HO and I. E. MELHUS. In May, 1936, it was observed that the stand of barley was unusually thin in many fields in central Iowa and that the plants were yellow and stunted. Some were flaccid and dying; at heading time the height of the grain was very uneven. The stunted plants headed short and were conspicuously yellow in contrast to the dark green healthy plants. A species of *Pythium* was isolated from the infected roots, which was identified as *P. graminicolum* and shown to be the causal agent of the disease. Cross inoculation indicated that timothy, millet, wheat, rye, and maize were actively parasitized, while oats and sorghum were much more resistant. *P. graminicolum* grew better in steamed alkaline soil than in acid soil (range of pH 4 to 11). The hyphae penetrated the root tips in the zone just back of the cap, moving inter- and intracellularly. Sex organs developed profusely in the parenchymatous tissue and vascular bundles of the young infected roots. Marked root necrosis and stunting of the aerial parts developed on seedlings grown in association with the pathogen in glass cylinders partially filled with steamed soil.

Strains of Tobacco Resistant to Tobacco Mosaic. FRANCIS O. HOLMES. A dominant gene, inducing a necrotic type of response to infection with tobacco-mosaic virus, has been transferred from *Nicotiana glutinosa* to *N. tabacum* through the intermediacy of the amphidiploid species *N. digluta*. After hybridization, successive backcrosses to *N. tabacum* combined with the selection of necrotic-type individuals produced *tabacum*-like plants which, when infected, had the disease manifestations characteristic of *N. glutinosa*. Repeated self-pollinations gave rise to wholly necrotic-type strains of tobacco. In the presence of the gene conferring the necrotic type of response, transfer of virus from infected plants is difficult. It is believed that tobacco-mosaic virus will be unable to maintain itself in the field in tobacco plants bearing this gene.

The Lime Factor in Bordeaux Injury. JAMES G. HORSFALL and R. F. SUIT. It was early established that copper in Bordeaux injures plants. The injury was lessened, never quite eliminated, but sometimes accentuated by increasing the lime. The accentuation was due to the lime itself on such lime-sensitive plants as the Solanaceae, Cucurbitaceae, hops, ginseng, roses, and others. Correct reply to the following questions will enable one to determine lime injury: Does lime alone produce it? Does reduced lime reduce it? Do ''insoluble'' coppers, minus lime, produce it? Lime damage is easily reproducible in the greenhouse, copper injury is not. Damage is favored by high temperatures on spray date; sprays are more injurious than dusts. Damage is reduced by lime carbonation or by antagonism of magnesium. Lime injury shows externally as a leaf deformation and accelerated transpiration; internally, as stunting and crisping of plant organs. The surface effects apparently result from a dissolution of cuticle, soluble in warm alkalis. Excess lime-Bordeaux or lime applied in water on a hot day furnishes ideal conditions for dissolution of cuticle, penetration of calcium, and increased water loss. Carbonation of the lime, or reduction in the concentration, would reduce the alkalinity and, simultaneously, the injury. Stunting and crisping aspects of lime damage probably result from a hardening of the cell walls by the calcium, so that they do not expand normally.

Soil Sterilization by Chloropicrin for Field Crops and Vegetables. FRANK L. HOWARD.

Effect of Growth-Inhibiting Suubstances Present in Nutrient Solutions of Aspergillus Species. WM. G. HOYMAN. Four species of *Aspergillus* were grown on Czapek's solution at room temperature for 15 weeks. The solutions were then filtered and autoclaved. The growth of each species was measured on potato-dextrose agar containing 10 ml. of filtrate. The toxic substance obtained from *Aspergillus niger* did not inhibit its own growth but reduced that of *A. terreus* approximately 90 per cent, *A. ochraceous* 40 per cent, and *A. wentii* 50 per cent. *A. ochraceous* did not produce a substance inhibiting the growth of the three other species, but its own growth was about 70 per cent that of the check. The filtrate of *A. terreus* inhibited the growth of that species, as well as that of *A. wentii* and

A. niger, approximately 50 per cent. That of *A. ochraceous* was retarded about 60 per cent. The filtrate of *A. wentii* checked the growth of *A. terreus* for 7 days, while the growth of *A. ochraceous* was 40 per cent that of the check. No effect was evident on the growth of *A. niger*, but its own growth was retarded about 40 per cent. It is evident that a thermostable growth-inhibiting substance is present in the filtrate of each species and is nonspecific in 3 instances.

Relation of Upper-air-mass Movement to Incidence of Stem Rust. H. B. HUMPHREY. An analysis of any major epidemic of stem rust and an interpretation of the rôle of weather in inducing it require consideration of high-elevation winds. Examination of weather data for the period May 15 to June 30, 1935, shows that at 4,000 ± feet the air-mass movement was often westward to southward to Oklahoma and Texas and thence sometimes northward. Such winds, carrying spores from New York, Pennsylvania, and the Great Lakes States, may have transported initial inoculum into Texas, Oklahoma, and Kansas. Weather data for May 1 to June 30, 1937, show a north-to-south high-elevation (4,000 ±) wind movement for a total of 18 days and of 43 days from south to north. On 29 out of 43 days the winds moved northward to Kansas, thence across Missouri and eastward. This preliminary study suggests the probable importance of the barberry in the North-Northeastern States to incidence of rust in the Midwestern States. To make a more comprehensive analysis of upper-air movement and its importance in transporting rust inoculum, it is proposed to release at stated times and places enough cornstarch to indicate the course of such winds and the ultimate disposition of their spore and dust content, caught by spore traps.

The Effect of Several Sulphur Sprays on the Photosynthesis of Apple Leaves in a Controlled Environment. RUSSELL A. HYRE. These studies were made on McIntosh and Baldwin apple leaves at temperatures of 70°, 85°, and 100° F., relative humidity of 60 per cent, and with the light provided by a unit of 4 10,000-lumen sodium vapor lamps. During 32½-hour periods (after spraying *vs.* before spraying) lime-sulphur solution, 2–100, gave about twice the reduction in photosynthesis as did Magnetic Spray Wettable Sulphur and Micronized Wettable Sulfur, both at 6 lbs. per 100 gallons. The reduction was about the same at 70° and at 85° but increased markedly at 100°. (At 85° the approximate reductions were: lime sulphur, 18½ per cent; Magnetic Spray Wettable Sulphur, 7½ per cent; Micronized Wettable Sulfur, 7½ per cent, while the check increased 3½ per cent. At 100° the reductions were about 59, 34½, 34½ and 12½ per cent, respectively.) During a 26-hour period, at 85° F., lime-sulphur solution, 2½–100, with and without arsenate of lead at 3 lbs. per 100 gallons, gave essentially the same reduction, whether applied to both surfaces of the leaf or to the lower surface only. When applied to the upper surface only, the reduction was slight. It seems probable that the marked increased in injury from lime sulphur on apple foliage, noted in recent years, may be due to the change in method of application; *i.e.*, from overhead applications with nozzles on spray rods to heavy driving applications from the ground with spray guns.

Further Determinations of the Carbohydrate-nitrogen, Relationship and Carotene in Leaf-hopper-yellow and Green Alfalfa. H. W. JOHNSON. Leaf-hopper-yellow alfalfa leaves, whether from infested cages or non-dusted plots, are higher in dry matter, reducing sugar, total sugar, and total acid-hydrolyzable substances, but are lower in total nitrogen than are green alfalfa leaves from noninfested cages or dusted plots. On the other hand, stems of leaf-hopper-yellow alfalfa plants from non-dusted plots are lower in dry matter, reducing sugar, total sugar, and total acid-hydrolyzable substances, but are higher in total nitrogen than are stems of green plants from dusted plots. The congestion of carbohydrates in the leaves and the deficiency of these substances in the stems of alfalfa plants yellowed by the potato leaf hopper, *Empoasca fabae*, are considered further evidence that the injury caused by this insect is due to a clogging of the food-conducting elements, with resultant interference with the translocation of elaborated food in the infested plants. In 1937, leaf-hopper-yellow, second-cutting Grimm alfalfa (leaves and stems) from an uncaged area contained only 71 milligrams of carotene per kilogram of dry matter, whereas green alfalfa from a caked area in the same small plot contained 227 milligrams per kilogram. The yellowed alfalfa is, therefore, only about one-third as rich in potential vitamin A activity.

Crotalaria Mosaic. H. W. JOHNSON and C. L. LEFEBVRE. A disease characterized by a general stunting of the plants, by mottling, blistering, and malformation of the leaves, and by abnormally stimulated lateral branching or witch's brooming was prevalent in the Crotalaria nursery at Arlington Experiment Farm, Arlington, Virginia, in 1937. The percentage of diseased plants in one or two rod rows of the *Crotalaria* species present was as follows: *striata* 86.7, *usaramoensis* 76.2, *intermedia* 71.6, *maxillaris* 58.1, *incana* 57.3, *spectabilis* (early strain), 46.2, *spectabilis* (late strain) 33.3, *lanceolata* 31.8, and *retusa*

0.00. From 50 to 67 per cent positive transmission of the virus from *C. spectabilis* and *C. usaramoensis* to healthy *Vicia faba* plants was obtained by rubbing the leaves with cheesecloth soaked in extract from mosaic plants, using white quartz sand as an abrasive. Definite mosaic symptoms were evident in 14 days. Eight noninoculated plants remained healthy. Witch's broom or curly disease of *C. anagyroides* and *C. juncea* was reported from Java in 1927 by M. B. Schwarz, who suggested that this might be a virosis spread by insects. The present report is thought to be the first record of the occurrence of this disease in the United States.

Brown Root Rot of Tobacco. JAMES JOHNSON. The true nature of brown root rot has remained obscure for 20 years. Fortunately, the disease may be prevented in field practice by avoiding certain preceding crops. No parasite has been conclusively and regularly associated with the disease. Histological studies reveal no organism in the diseased tissues. Diseased cells are brown and sharply delimited from normal cells. The cell walls are thickened and the cell contents granular before complete disintegration. The lesions are generally limited to the cortex, lateral progress of the disease being effectively checked in the endodermal region. Studies on the properties of the causal agent have shown that it is destroyed by drying. Temperatures of 45° C. for one day, freezing below −10° C. for several days, charcoal and many chemical disinfectants also eliminate the causal agent from the soil. Air, pure oxygen, or light has little or no effect. Diseased root pulp reproduces the disease, but filtered extracts of diseased roots or water extracts of the soil do not. The possible relation of toxins produced by soil saprophytes is being investigated further, but no conclusion has been reached as to the true nature of the causal agent.

Crinkle and Mosaic of Geranium. LEON K. JONES. Two viroses of geranium, *Pelargonium zonale*, observed in greenhouses in the State of Washington, have been designated as crinkle and mosaic. Both viruses are transmissible to geranium by grafting but nontransmissible to geranium, tobacco, and tomato by mechanical inoculation. Crinkle is characterized by development of irregular to circular, chlorotic areas, one-half to 2 mm. in diameter, in the leaf tissue; ruffling, dwarfing, yellowing and dropping of foliage; dwarfing of plants during the midwinter months; and masking of symptoms during the summer months. Mosaic is characterized by mottling of foliage with light and dark green areas, dwarfing of leaves, and shortening of stem internodes. The light green areas are usually large, irregular, and interveinal.

The Resistance of Katahdin Potato Seedlings to Infection by the Veinbanding Virus and the Tobacco Mosaic Virus. LEON K. JONES and EARL F. BURK. Tests in the greenhouse with seedlings produced from an Early Northern × McCormick cross (200–5) and from Katahdin selfed have shown that the Katahdin selfed seedlings carried considerable resistance to infection by the veinbanding virus, but the 200–5 seedlings were very susceptible; and that the symptoms in the affected Katahdin selfed seedlings varied from mottling to severe necrosis, but the symptoms in each of the affected 200–5 seedlings were characterized by mottling without necrosis. Similar results were observed from natural infection in the field, except that percentage of infection was higher than that obtained by mechanical inoculation in the greenhouse. Seedlings produced from a 200–5 × Katahdin cross showed a lesser degree of resistance to natural infection by the veinbanding virus that the Katahdin selfed seedlings but a slightly greater tendency towards necrosis in affected plants. Mechanical inoculation with common tobacco mosaic (tobacco virus 1) into Katahdin selfed and 200–5 seedlings in the greenhouse showed that Katahdin selfed seedlings were immune from and 200–5 seedlings were somewhat susceptible (8.3 per cent) to infection; and that symptoms in affected 200–5 seedlings varied from severe necrosis to mottling without necrosis.

The Value of Some New Copper Fungicides for Apple Blotch (Phyllosticta solitaria) Control. K. J. KADOW and H. W. ANDERSON. All the new fungicides intended as substitutes for Bordeaux mixture, which could be secured readily, were tested in an experimental way as possible apple-blotch sprays. With few exceptions, the materials were used as recommended. Those tested were Bordeaux mixture, Bordeaux 34 with zinc sulphate and lime, Oxo Bordeaux, Basicop, Coposil, Copper Zeolite, copper phosphate with lime and bentonite, Cupro K, Cuprocide 54, 66a, Copper-Hydro 40, and a special copper from the Niagara Sprayer and Chemical Company. The sprays were applied to specially selected Duchess trees, and the season was ideal at Urbana, Illinois, for the development of blotch and spray injury. Of all materials used, the only ones that controlled blotch and, at the same time, did not cause commercially important spray injury were (concentrations in 100 gallons of water): Bordeaux 34 (2 pounds) with zinc sulphate (¾ pound) and lime (¾ pound); Oxo Bordeaux (6 pounds), Copper Zeolite (3 pounds); Copper-Hydro 40 (3 pounds); and Cupro K (3 pounds). Copper phosphate (4 pounds), used

with 6 or 8 pounds of lime and 4 pounds of bentonite, may also be satisfactory, since, at one-half that strength, the spray controlled all but petiole infections, without spray injury. Cupro K caused slight specking on some fruit.

Heterothallism and Segregation for Pathogenicity in Venturia inaequalis. G. W. KEITT, D. H. PALMITER, and M. H. LANGFORD. In 1936, using cultures and methods previously reported (Science, n.s., 85: 498), 8 monoascosporic isolates from an ascus were inoculated separately and in all possible pairings on apple leaves, which, with controls, were overwintered and examined for perithecia. Seven of the 36 inoculated samples disintegrated. Data from the others, confirming previous results, indicate that the isolates comprise 2 groups of 4 each, being hermaphroditic, self-sterile, intra-group sterile, and inter-group fertile. All 14 available samples from the 16 expectedly fertile pairings, and none other, yielded mature ascocarps. Singly and in all possible pairings the same 8 isolates were cultured on cheesecloth "wicks" in bottles containing nutrient solutions. Perithecial initials were formed in all cases. They grew larger in expectedly fertile pairings than others. Mature ascospores were produced only from the 4 inter group pairings involving isolate 8. On leaves of differential apple varieties 4 of the 8 isolates were typically pathogenic; 4 induced only flecking. Two isolates of each fertility group comprised, each 4, of like pathogenicity. Inoculation experiments with cultures from ascospores of known serial arrangement in the asci showed that segregation for pathogenicity occurred alternatively in the first or the second nuclear division in the ascus.

A Seed-borne Bacterial Disease of Garden Stocks, Matthiola incana. JAMES B. KENDRICK. A serious bacterial disease of garden stocks has been present in commercial plantings in certain coastal sections of California since 1933. On seedlings the first evidence of the disease is a soft, watersoaked condition of the stem and the collapse of the plant. Older, more woody plants show large, irregular, oblong to linear, watersoaked areas on the stem and branches that later become sunken, dark brown lesions. Complete girdling of the main stem or branches often results in the death of the plant or the affected branches. Isolations and pathogenicity studies have shown that a yellow *Bacterium* is the cause of the disease. The organism closely resembles *B. campestre* in morphologic and cultural characters, but reciprocal cross inoculations have failed. Seed from diseased plants grown in sterile soil has shown the causal organism to be seed-borne. The organism has been cultured from the seed. Microtome sections also have shown the bacteria in the seed coat and funiculus. Effective control has been secured by hot-water treatment of the seed for 10 minutes at 53° C.

The Inhibiting Substance Formed by Diplodia zeae. G. C. KENT. A cultural study on the nature of the inhibiting action of filtrates of *Diplodia zeae* on the growth of mycelium of this species has been carried out. The inhibitor was obtained after 35 to 42 days' growth on a modified Czapek's solution and withstood dilution with distilled water up to 1–125. The inhibitive action was not destroyed by oxidation, by correction of hydrogen-ion concentration, replacement of sugar in the cultural medium, moderate hydrolysis under acid or alkaline conditions, or by boiling for 1 hour. The alkaline nature, insolubility in ether, and nonvolatile nature of the inhibitive material, coupled with the above data, are believed to indicate the presence of one or more complex nitrogen containing compounds. This inhibitor has been obtained by extracting various modifications of Czapek's medium, sterilized grain media, and corn plants growing in the field, all of which have supported the growth of the organism.

The Relative Effect of Genetic and Environmental Factors on Growth Types of Ustilago zeae. M. F. KERNKAMP. When sporidia are isolated from many promycelia of *Ustilago zeae*, the resulting cultures are usually sporidial, at first. Some change promptly to mycelial; some remain sporidial for varying periods of time, then become intermediate or mycelial; others remain sporidial indefinitely. As the factors affecting these changes were not known, sporidial, mycelial, and intermediate cultures were grown under varying environmental conditions. It was found that the intermediate lines varied considerably with certain environments, whereas mycelial and sporidial lines remained relatively constant. Chlamydospores resulting from crossing sporidial and mycelial lines gave rise to promycelia on which sporidia and hyphal branches were produced in the following ratios: 4: 0, 3: 1, 2: 2, and 1: 2: 1. When chlamydospores of this cross produced 4 sporidia it was possible to isolate all 4 and grow them in culture. These produced either sporidial or mycelial growth in the same ratios as given above. The results show that the growth types are determined genetically and that the environment influences intermediate lines considerably, although it has no appreciable effect on sporidial or mycelial lines.

Effect of Prolonged Storage of Treated Seed Corn. BENJAMIN KOEHLER. Seed corn was treated with Barbak III, Merko, and Semesan Jr. (Cresol mercury 1929–32, ethyl mercury phosphate 1933–37), so as to give a storage period of 3 months as compared with only 1 day before planting in a 5-year test. In another test, running 4 years, seed was held over for 1 year, some being treated a year before planting and some not until immediately before planting. All tests were made with seed having a moisture content of 12 per cent or less. When storage was in a warm building or in a closed, unheated warehouse, the yields of plants grown from stored treated seed were frequently as good as or better than those from freshly treated seed. There were, however, some cases of seed injury. On the whole, the freshly treated seed gave the better performance by a small margin. When storage of treated seed was up under the roof of an open shed, there was definite injury to the seed in every case. The several mercurials used gave somewhat similar results with respect to the effect of storage on treated seed.

Seed Treatment Tests with Crown-injured Corn. BENJAMIN KOEHLER. During the past 2 years crown-injured seed corn has been included in the routine testing of commercial seed disinfectants at the Illinois Station as an accelerated method of testing the protective properties of disinfectants against soil-borne organisms. Parallel treatments also were made with good seed and Diplodia-infected seed, both with sound seed coats. Yield of grain from crown-injured nontreated seed corn has been from 20 to 35 per cent below that from grain similarly injured, but treated with some of the better disinfectants. Red copper oxide was one of the best disinfectants for protecting the seed against soil-borne infection. It was slightly better than the mercurials, perhaps, through greater safety for the sprouting embryo. In the control of Diplodia seedling disease, however, certain mercurials were in the lead, red copper oxide being only 59 per cent as good as the best of these. Of 2 new proprietary compounds, one gave partial control against soil-borne infection but none against Diplodia, while the other behaved in the opposite manner, giving partial control of Diplodia, but, apparently, because of toxicity, it gave only a decrease in yield when applied to crown-injured seed.

Properties and Host Range of a Cabbage Mosaic Virus. R. H. LARSON and J. C. WALKER. The virus of cabbage mosaic found in southeastern Wisconsin appears to differ from those viruses previously described. Shepherd's purse, *Capsella bursa-pastoris*, and penny cress, *Thlaspi arvense*, were proved to be natural overwintering hosts. *Myzus persicae* and *Brevicoryne brassicae* are vectors. The virus is inactivated at 55° C. for 10 minutes; it remains infectious *in vitro* for 2 days at 20° to 22° C. and when diluted up to 1 to 1000. Systemic infection was obtained on all cultivated and wild species of *Brassica* tested, on dame's violet, *Hesperis matronalis*, and on wallflower, *Cheiranthus allonii*. On stock, *Mathiola incana*, infection was followed by breaking of the flowers and variegation of the petals. Systemic infection also was obtained on spinach, Swiss chard, sugar beet, zinnia, and calendula. Local lesions were produced on *Nicotiana tabacum* (var. Conn. Havana No. 38), *N. calyciflora*, *N. sylvestris*, and the F_1 hybrid of *N. tabacum* × *N. glutinosa*; local lesions followed by systemic infections occurred on *N. repanda*; systemic infection occurred on *N. bigloweii*, *N. glutinosa*, *N. langsdorfii*, *N. muelivalvis*, *N. quadrivalvis*, and *N. rustica*.

A Potato Wilt Caused by the Tarnished Plant Bug, Lygus pratensis L. J. G. LEACH and PHARES DECKER. A destructive wilt of potatoes observed in Minnesota since 1935 resembles Fusarium wilt, although the symptoms are not typical. Repeated inoculations in field and greenhouse with Fusaria isolated from diseased plants failed to produce the disease. Field experiments with hill- and tuber-unit plots indicate that the disease is not tuber-transmitted, and the prevalence of the disease on virgin soil planted with selected healthy tubers indicates that it is not soil-transmitted. The disease is not appreciably influenced by fertilization. Tarnished plant bugs were abundant in affected potato fields, and, when nymphs and adults were allowed to feed for approximately 2 weeks on the stems of potato plants in the greenhouse, a characteristic wilt was produced. About 3 weeks after the bugs were removed a marked chlorosis appeared on the terminal leaves, although the bugs fed only on the stems. Only the stems on which the bugs fed were affected. The stems wilted about 10 days after the apparance of the first symptoms on the leaves. The symptoms were not identical with those observed in the field but may be comparable when due allowance is made for the effect of the greenhouse environment. (Cooperative investigations between the Division of Plant Pathology and Entomology, University of Minnesota.)

A Practical Solution of the Sclerotium rolfsii Problem in the Beet-sugar Industry. LYSLE D. LEACH and JAMES B. KENDRICK. *Sclerotium rolfsii*, first observed in 1931, was

then believed to be a serious threat to the beet-sugar industry of central California. The loss in 1933 was about 5,000 tons and the known infested acreage increased from 4,600 in 1932 to 17,600 in 1936. The spread of the fungus has been checked by proper handling of screenings from beet loading stations, wash-water from sugar factories, agricultural machinery, and livestock from infested fields. A method of soil sampling to determine the population of viable sclerotia has been developed. The volume of inoculum in the soil increases rapidly with each crop of beets and decreases in seasons when certain other crops are grown. By proper crop rotation, the soil inoculum may be reduced to the point where beets may safely be grown again within 3 to 5 years. Fields known to be infested are being indexed annually by the soil-sampling method, and the incidence of infection rather accurately predicted. Infection may be distinctly reduced by the application of nitrogenous fertilizers. During 1936 and 1937, there has been a definite decrease in the rate of spread of the fungus and a very great decrease in the occurrence of severe losses.

Ruffle Leaf of Tobacco. S. G. LEHMAN. In August, 1937, many tobacco plants showing striking leaf malformations were found near Raleigh, North Carolina. On affected plants leaf-blade development is repressed in varying degrees causing some leaves to appear petiolate, others string-like. Leaf margins turn downward in a narrow tight roll, while the blade as a whole may fold upward along the midrib. Veins frequently coalesce, binding and making the margin appear crenate. V-shape accessory laminae, "Ruffles," grow downward from the lower side of leaves whose blades are not too strongly repressed. Neither chlorosis nor necrosis occurs, but diseased leaves usually appear greener and thicker than normal leaves. Stem elongation usually is not repressed, nor do axillary buds show abnormal tendency to grow into suckers. Accessory flower parts develop. Affected plants set seed abundantly. Attempts to transfer the malady by mechanical inoculation, by insect vectors, and by grafting have given only negative results.

The Source of Nitrogen and Accessory Growth Factors in the Nutrition of Some Fungi. LEON H. LEONIAN and VIRGIL GREENE LILLY. According to their ability to utilize different sources of nitrogen, some 40 representative fungi were divided into the following groups: 1. Those utilizing the nitrogen of ammonium salts, of nitrates, and of amino acids without the aid of vitamin B_1. 2. Those utilizing ammonium and amino acid nitrogen but not nitrate nitrogen. 3. Those utilizing amino acid nitrogen but not ammonium nitrogen. 4. Those utilizing either ammonium or amino acid nitrogen but only in the presence of vitamin B_1. (1 p.p. 20 million.) 5. Those capable of using amino acid nitrogen but not ammonium or nitrate nitrogen in the presence of vitamin B_1. 6. Those that seemed incapable of utilizing any of the foregoing sources of nitrogen, either in presence or absence of vitamin B_1. Fractionation of yeast extract has yielded a concentrate that induced good growth in this and all groups of fungi tested. Pure crystals of vitamin B_2 (lactoflavin) could not replace vitamin B_1 and manifested a comparatively slight catalytic property. Although vitamin B_1 is a controlling factor in the growth of several fungi, under certain conditions it was replaced by substances devoid of vitamin B_1. Also, it seems possible that the essential amino acids may be replaced by other materials whose exact nature is still unknown. Whether such substances consist of amino acids in combined form, or contain some catalytic materials other than the known vitamins, remains to be determined.

Potential Agents of Biological Control of Plant-parasitic Nematodes. M. B. LINFORD and J. M. OLIVEIRA. Soil populations of plant-parasitic nematodes are influenced by an unstable, interacting complex of diverse parasites and predators. Recognition of components and partial analysis of their interactions have been facilitated by transparent, low-nutrient media containing nematodes. By sampling chiefly roots and soil from Hawaiian fields and gardens infested with the root-knot nematode (*Heterodera marioni*), the writers have, since October, 1935, collected 52 enemies of this pathogen, most of them attacking also *Pratylenchus pratensis* and many other nematodes, and some attacking one another. All but *Mononchus* spp. and one mite, in the following list, have been new records for Hawaii: Eleven nematode-trapping fungi (Phycomycetes and Hyphomycetes), 1 egg parasite (*Penicillium* sp.); 6 non-trapping parasites (*Chytridiales, Ancylistales,* and Hyphomycetes); 1 ecto-parasitic protozoan; 24 predacious nematodes (5 *Aphelenchoides,* 2 *Diplogaster,* 14 *Dorylaimidae,* and 3 *Mononchus*); 6 mites; and 3 predacious tardigrades (*Macrobiotus*). Some of these have been found in sand, volcanic ash, forest litter, and soil from sea level to 9,700 feet, but, where *H. marioni* is established, several are wide spread and abundant; 18 species have been collected within half a mile. Normal activities of these agents restrict nematode populations, but practical biological control awaits means of increasing these activities.

Root-knot Injury Restricted by a Nematode-trapping Fungus. M. B. LINFORD and FRANCIS YAP. An experiment, involving 10 treatments of 10 5-gallon pots each, compared

plant growth during 15 months in steamed soil as follows: A and B, without nematodes; C and D, with *Heterodera marioni*; and E and J, with nematodes plus 6 isolates of nema-trapping Hyphomycetes grown in a nutrient-bagasse medium. Sterile bagasse medium was added to B and D; *H. marioni* larvae were added, 490 per pot, to all but A and B; and these two were infested with desiccation-tolerant flora and fauna separated from the nematode suspension. Nematode infestation reduced pineapple top growth as follows: 40 per cent without bagasse, 34 per cent with bagasse, and only 28 per cent with bagasse plus *Dactylella ellipsospora*. Reductions in total root length were 73, 69, and 57 per cent, respectively. *Arthrobotrys oligospora* (2 isolates), *A. musiformis*, *Dactylella* sp., and *Dactylaria thaumasia* were without apparent effect. Chance infestations of an egg parasite (*Penicillium* sp.) accompanied *H. marioni* throughout and, among C and D plants, 14 of 17 sampled were contaminated with trapping fungi; consequently, the full degree of biological control was not measurable. Reisolations from galled roots showed that species of trappers, introduced experimentally, remained dominant during 15 months.

Growth Tests with Extracts of Erwinia amylovora, Phytomonas rhizogenes, Taphrina cerasi, T. deformans, and Ustilago zeae. GEORGE K. K. LINK, HAZEL W. WILCOX, and VIRGINIA EGGERS. Dextrose-tryptone, and dextrose-tryptone-peptone broth cultures of each of these organisms were adjusted to pH 2.5 with 2N HCl and filtered through pyrex wool. Glass and residue were frozen with solid CO_2, ground, while frozen, and added to the filtrate. Each lot was divided into 125 cc. samples. Each sample was extracted for 9 hours with peroxide-free ether in an apparatus devised by Gallagher, Koch, and Dorfman for quantitative extraction of sex hormones from small samples of urine. Extraction by shaking with ether also was tried. Crude extracts of each lot were tested for auxones by application to intact coleoptiles of Avena and to intact hypocotyls and internodes of *Phaseolus vulgaris*. Extracts obtained by shaking were found inactive. Those obtained by use of the extractor produced growth responses in the treated plants. These results warrant the conclusion that each organism tested produces one or more auxones and that these may be parts of chemical mechanisms by which these parasites disturb growth of their hosts. The Salkowski test indicates β-indoleacetic acid as a constituent of each extract.

Growth Substance in Crown Gall. S. B. LOCKE, A. J. RIKER, and B. M. DUGGAR. Responses of inoculated plants, such as epinasty of petioles, increased initiation of adventitious roots, increased cambial activity, suppressed development of axillary and adventitious buds, and delayed abscission of petioles, which indicate the presence of growth substances, were induced to a greater degree by a virulent culture of *Phytomonas tumefaciens* than by an attenuated sister single-cell culture. The attenuated culture stimulated the development of adventitious shoots. Tomato stems inoculated with the virulent culture yielded, during at least 10 days immediately following inoculation, more growth substance than comparable noninoculated tissue, as measured by Went's diffusion method. Three per cent beta-indole-acetic acid in lanolin, applied to the tips of decapitated tomato stems, was less effective than substances diffusing from galls induced by the virulent culture in stimulating proliferation about inoculations with the attenuated culture in the stem below. Efforts to differentiate virulent and attenuated cultures on the basis of growth substance produced in culture have thus far been unsuccessful. The similar growth rates, based on frequent counts of virulent and attenuated cultures in tomato stems, suggest that there is no selective bacteriostatic action of the host.

Influence of Temperature and Humidity on Powdery Mildew of Roses. KARLA LONGRÉE. Temperature studies on conidia on rose leaves indicated for germination: minimum 3°–5° C., optimum 18°–24° C., maximum 33° C.; for mycelial development: minimum 6° C., optimum 18°–24° C., maximum 27° C.; for sporulation: minimum 9° C., optimum 18°–24° C., maximum 27° C. At temperatures just above the optimum, spores mature and age rapidly; below, maturing is slow and germinability preserved longer. Detached spores die readily with increased temperatures and decreased humidities. Free water decreases germination. Great difficulties were experienced in obtaining relative humidities close to 100 per cent without getting condensation, but this was finally accomplished through precise temperature control. It was found that the relative humidity need not be higher than about 97 per cent in order to give excellent germination but that, above and below 95 per cent, germination is close to zero and ceases below 75 per cent. In comparing germination of conidia on glass slides and on leaves of rose shoots, kept also under controlled conditions, it was concluded that the relative humidity at the surface of young rose leaves is very high, even in a dry atmosphere.

Phycomycetes in Iowa Soil. CLIFFORD H. MEREDITH. An examination of 20 types of cultivated soils in the northern, central, and southern parts of Iowa revealed the pres-

ence of many rapid-growing, nonseptate fungi. These were obtained from 1 g. lumps of soil placed in a Petri dish of plain agar. The tip of the most rapid-growing organism was freed from bacteria by placing it under plain agar in a plate and taking the fungus off when it grew to the surface. Six of these were identified as *Pythium de baryanum*, *P. pulchrum*, *P. rostratum*, *P. ultimum*, *P. graminicolum*, and *Aphanomyces* sp. In April and May, 1936, species of *Pythium* predominated in the soils under observation. In July and August fewer isolates of phycomycetous fungi were obtained. In April, 1937, 10 isolations from each of 5 soils yielded 50 cultures of Phycomycetes, with *Pythium de baryanum* predominating. Isolations made from soils in corn fields the first of July yielded a high percentage of pythiaceous fungi; while isolations made the last of July, following a hot, dry period, gave few species of *Pythium*.

Fusarium avenaceum, a Vascular Parasite of Potato. JOHN G. MCLEAN. A disease of potato caused by *Fusarium avenaceum* was first observed in Wisconsin in the summer of 1936 and has since been collected throughout the State. The fungus is definitely pathogenic, penetrating the roots and producing a seed-piece decay. Symptoms vary with time of infection, soil and air temperature, and soil moisture. Tuber symptoms resemble those of the more common potato wilts. Top symptoms differ from those produced by *F. solani* v. *cumartii* and *F. oxysporum*. There is a reduction of leaf area due to the decrease in size of the leaflets on apical leaves and an increase in the number of folioles. The axillary buds are stimulated into production of secondary shoots or aerial tubers. Chlorosis and tipburn are typical, along with rigidity of stem and petiole. The leaves roll upward and assume a harsh texture. Reddening or purpling of the tops is not uncommon. Cobblers, Triumphs, Katahdins, and Rurals are susceptible. Early varieties are more apt to escape the disease.

The Relation of Nitrogen Nutrition to Virulence in Phytomonas stewarti. GEORGE L. MCNEW. Ten strains of *Phytomonas stewarti* that differed in virulence were tested for differences in physiology, including ability to use inorganic nitrogen. Virulent cultures grew readily when seeded on an agar medium containing ammonium nitrate as the only source of nitrogen, but weakly virulent ones failed to produce visible colonies unless organic nitrogen was added. Of 510 single-colony isolates that were seeded on the synthetic medium, all the virulent ones grew readily; but many of the weakly virulent ones grew sparsely, if at all. When these weakly virulent cultures were induced to grow on the medium, they gained in virulence. Conversely, if the cultures were restored to virulence by host passage, they gained ability to use inorganic nitrogen. Of the 10 original strains tested, only the most virulent one reduced the nitrates in the synthetic medium to nitrites. Nitrites were also identified in wilted plants inoculated with either this or other highly virulent cultures. As has been demonstrated for other wilt diseases, nitrite solutions caused wilting when injected into healthy plants. However, *Phytomonas stewarti* may cause severe wilting without producing nitrites when inoculated plants grown in sand are deprived of nitrates and supplied only with ammonium nitrogen.

Distribution of Bacterial Cells in Poured Plates. GEORGE L. MCNEW. In order to determine if the dilution-plate method is reliable for securing single-cell cultures, observations were made on the distribution and growth of bacterial cells in agar. Agar from dilution plates seeded with broth cultures was sliced, stained, and examined under oil immersion. In plates inoculated with *Phytomonas stewarti*, 99.0 ± 0.35 per cent of the points occupied by the bacteria were seeded by single cells. The isolated cells grew readily when incubated under aseptic conditions. Most of them divided within 3 hours and their progeny about every 1½ hours thereafter. In 2 preparations only 6.5 and 11.3 per cent of the single cells failed to divide within 22 hours. Similar results were secured with 3 other strains of *Phytomonas stewarti* and with *P. translucens* var. *undulosum*, *P. campestris*, *P. phaseoli*, *P. angulatum*, *P. savastanoi*, *P. pisi*, *P. tabacum*, *P. pruni*, and *Erwinia carotovora*. The percentage of single cells was lower (90 to 97) with *P. michiganensis*, *P. juglandis*, *P. tumefaciens* (3 stains), and *P. insidiosum*. *P. fascians* was the only species in which clumping of cells occurred extensively. There was no evidence that the melted agar caused agglutination, since the clumps existed in culture before the bacteria were suspended in agar.

A New Race of Crown Rust That Attacks Bond Oats. M. B. MOORE, A. R. DOWNIE, and H. C. MURPHY. An apparently new race of *Puccinia coronata avenae* has been found that differs from Murphy's race 1 in that Bond, a variety used extensively in breeding for crown-rust resistance, is susceptible. Two collections were made in the spring of 1937 in Texas. These collections, together with race 1, previously obtained from H. C. Murphy, were increased in the greenhouse and used for inoculation in the oat-breeding nursery at University Farm. Subsequently, an epidemic of crown rust developed on Bond and its

hybrids. The Bond race may have originated in either of the two Texas collections or as a mutant or mixture in the material obtained from Murphy.

Fusarium Yellows of Gladiolus. RAY NELSON. A destructive disease of field plants and stored corms of gladiolus is apparently of general occurrence in the commercial producing sections of the United States and has recently attained major importance. Losses have been heavy in some areas, in some instances forcing the abandonment of large acreages of infested soil. The field aspects are those of a systemic vascular mycosis and include, most typically, dwarfing of the plant and progressive yellowing and dying of the leaves. Losses from decay in stored corms grown on infested soil have been excessive, exceeding $10,000 in one cellar in 1937. Various types of corm decay occur, including a circumscribed core rot and a spongy decay of the flesh that originates from infected vascular strands. Surface decay is usually initiated only at nodes where these strands terminate. The causal relation of a section Elegans *Fusarium* isolated from infected plants and corms has been established in numerous experiments. The temperature relations of the pathogen and the effects of temperature and moisture on the disease have been studied and are essentially like those reported in other fusarial mycoses. Varietal reactions vary from complete susceptibility to apparent immunity. Most of the leading commercial varieties are susceptible.

Dissemination and Viability of Conidia of Peronospora destructor. A. G. NEWHALL. Negative results in the control of onion mildew were obtained in 1937 by hot-water seed treatments in western New York. Field observations indicated distant sources of wind-borne inoculum. Surveys have shown that primary sources could be top-set or winter onions, often grown in small town gardens. Many such plantings were found sporulating in May several weeks before field infections on the commercial crops could be found (June–July). Wind-borne conidia were caught July 15 on slides exposed overnight ½ mile from the nearest onions. During an airplane flight, August 1, over infected onion fields, 132 conidia were caught on plain agar Petri-dish spore traps exposed at 6 levels up to 1500 feet, the upper limit of this test. Of these, 100 germinated normally in a few hours. That such spores are important in disseminating mildew is indicated by laboratory tests, which have shown some conidia can remain viable on glass slides held for 3 days at 70 per cent relative humidity and 15° C. and for 5 days at 9° C. Some germinated after being frozen 15 hours. They withstood 7 hours' exposure to September sunshine in drops of cold water. Malachite green inhibited spore germination much better than copper sulphate.

The Application of Electrodialysis to the Study of Copper Fungicides. A. A. NIKITIN. A study was made to correlate the chemical composition to the behavior of the copper compounds when used as fungicides. Electrodialysis methods as used in the study of soils were adapted to this purpose. From the character of a deposit, variation of current and temperature, certain deduction can be made concerning the behavior of the copper compounds for agricultural purposes. The rate of decomposition is related to the character and composition of the compound. This rate may be evaluated by observation of the change of pH in the cells, the time required for discoloration of the deposit, and the change in the character of the dispersed phase. By the use of electrodialysis it is possible to determine the effect of various acid radicles on the chemical behavior. The degree of discoloration of the deposit on the diaphragm has been found indicative of the character and composition. Furthermore, the adhesiveness and spreading properties of a fungicide can be, in most cases, estimated from the degree of adherence of the deposit to the diaphragm. It seems that electrodialysis helps to establish the facts of importance in understanding the action of copper fungicides.

Studies on the Efficiency of Colloidal Copper Fungicides. A. A. NIKITIN. The fungicidal efficiency of a copper compound depends upon interrelated chemical and physical properties. It has been found that the principal factor responsible for most of such efficiency is a colloidal state. For instance, colloidal-copper compounds, when applied to foliage, have shown little if any tendency to cake, scale, or wash off. Also, uniformity of composition may be most easily attained when the material is prepared as a colloid. Experiments have shown that the colloidal state, to have beneficial characteristics, must be formed in the initial precipitation of the fungicide. When it is formed by mechanical treatment of a crystalline phase or is induced by the addition of spreading agents, these properties are largely lacking. Studies on the compatibility of copper compounds with arsenicals with and without lime have shown that the colloidal state inhibits the chemical and physical changes that usually occur when the material is in the ordinary crystalline condition.

Differentiation of 5 Mosaic Viruses of Legumes. H. T. OSBORN. Pea virus 1, pea virus 2, vein mosaic of red clover, a virus from white clover, and a virus from white sweet

clover were studied in *Vicia faba* and other hosts. Certain distinctive characteristics serve to differentiate these viruses. Pea virus 1 causes enations to develop on pea and crimson clover. Transmission by the pea and potato aphids requires an incubation period in the vector. Pea virus 2 comprises a group of strains from which the Perfection variety of garden pea is immune. Transmission by known aphid vectors is without an incubation period. Vein mosaic of red clover is characterized by symptoms produced along the veins in several legumes. Transmission by the pea aphid is without an incubation period. The virus from white clover produces distinctive necrotic primary lesions on mung bean. The virus from white sweet clover differs from the 4 preceding viruses in that it is transmissible to solanaceous hosts. It causes necrotic primary lesions on bean, mung bean, and cowpea. Necrotic primary lesions are followed by systemic infection in *V. faba* and tomato. It differs from Price's ring spot 2 and a California strain of spotted wilt of tomato, which were transmitted to *V. faba* for comparison.

Soil Rot of Sweet Potatoes in Louisiana. L. H. PERSON. Soil rot of sweet potatoes, first noted in the Sunset sweet-potato district of Louisiana in 1934, has since increased in severity. In 1936 several fields were a total loss. During the growing season of 1937 a systematic survey of the sweet-potato area showed the disease much more widespread and destructive than was formerly thought. In some sections examined it was so severe that entire fields were abandoned. The symptoms are very characteristic, the plants being stunted, with small, thin, pale green to bronze leaves. The root system is badly rotted, and in heavily infected areas the plants fail to grow; many die before the end of the season. From 1 year's tests sulphur applied to the soil at the rate of 700 and 1,000 pounds per acre has given very promising results. Applications of 400 lbs. were ineffective. The pH of the soils varied from 5.7 to 6.0 before the sulphur was applied and dropped to 4.9 to 5.2 after sulphur applications. The yields per acre of grades 1 and 2 on duplicate plots were as follows: Check, 1.6 bushels; 400-pound rate, 21.0 bushels; 700-pound rate, 94.5 bushels; and 1,000-pound rate, 112.22 bushels.

Potato Spraying Experiments in Louisiana in 1936–1937. MILTON A. PETTY, JR.

The Effect of Flue-curing on the Survival of Ordinary Tobacco Virus 1. J. A. PINCKARD. Air and leaf temperatures at various points in 12 barns of flue-cured tobacco were studied in relation to the survival of the mosaic virus naturally present in the leaves. By frequent removal of standardized samples and their subsequent inoculation into tobacco plants, it was possible to determine the approximate temperature and exposure required to inactivate the virus. The virus survived the curing process throughout 4 of the first 5 barns cured and in the lower and middle tiers of the fifth barn. Temperatures of the lower tiers were from 191° to 196° F. for not more than 7 hours during final drying. In the remaining 7 barns, and even though final drying temperatures of 193° to 208° F. were maintained in the lower tiers for several hours, the virus was not inactivated in certain other portions of the barns where the temperatures varied as much as 50° F. These results show that the flue-curing process of primed tobacco is an uncertain means of virus inactivation and that flue-cured tobacco may become a dangerous source of primary infection if used by growers while preparing for succeeding crops.

Bacterial Wilt and Rot of Potatoes. H. N. RACICOT and D. B. O. SAVILE. Sets from diseased tubers planted in field plots, of which 75 per cent germinated, produced 79 per cent diseased plants. Sets from healthy tubers needle-inoculated around the eyes with a suspension of the causal organism, of which 98 per cent germinated, produced 97 per cent diseased plants. Sets with cut surfaces waxed, then dipped into suspension, of which 100 germinated, produced 34 per cent diseased plants. Sets cut with a knife purposely contaminated by cutting diseased tubers, of which 97 per cent. germinated, produced 97 per cent. diseased plants. Tubers dipped into suspension, dried, then cut into sets, of which 99 per cent grew, produced 54 per cent diseased plants. Tomatoes, eggplants, and peppers were successfully inoculated with the causal organism. The following did not become infected: *Solanum capsicastrum*, *S. dulcamara*, *Petunia hybrida*, *Nicotiana tabacum*, *Physalis pubescens* (husk tomato), and *P. heterophylla*.

Cell Stimulation by Chemicals in Relation to Crown Gall. A. J. RIKER and R. NAGY. A number of substances were mixed with lanolin and applied to decapitated test plants for determining their activity in stimulating cell growth. The substances included (1) living and lyophilized crown-gall bacteria and extracts from them; (2) culture filtrates and several metabolic fractions; (3) lyophilized tomato crown gall and lyophilized uninfected tomato stems; (4) beta-indole-acetic acid; (5) methyl cholanthracene and 1, 2, 5, 6 dibenzanthracene; and (6) several miscellaneous compounds, including vitamin B_1. (This last substance is produced from synthetic media by crown-gall bacteria in a quan-

tity comparable to that by yeast). Test plants included primarily bean and sunflower, but tomato, Coleus, and Impatiens also were employed. In general, the beans showed a distinct but variable response to many of the materials, especially those in groups (1) to (4), inclusive, while the sunflower reacted to very few. On beans 3 per cent beta-indole-acetic acid, renewed every week, induced the greatest proliferations, while, on the other plants, those from crown-gall inoculations were largest.

The Problem of Breeding Rice for Resistance to Cercospora oryzae. T. C. RYKER. About 75 per cent of the rice acerage in Louisiana is planted to the varieties Blue Rose and Early Prolific. These varieties are highly susceptible to Cercospora leaf spot, Ordinarily, Early Prolific, because of early maturity, is not seriously affected, while Blue Rose, which matures considerably later, is. All fields of the latter variety are usually rather uniformly infected. When the infection is severe a premature browning and dying of the plants occur. Certain commercial varieties, such as Rexora and Fortuna, are highly resistant, but even these tend to become susceptible when maturing. A study of the inheritance of resistance is complicated by the facts that all degrees of resistance from highly susceptible to highly resistant occur and that susceptibility varies with the maturity of the plants. A method of inoculation has been employed that apparently permits selection for resistance. Several selections made in Blue Rose in 1936, when tested in the field in 1927, appeared to be resistant.

Attenuation of Crown-gall Bacteria. A. J. RIKER, J. VAN LANEN, and I. L. BALDWIN. The bacteriological character responsible for the pathological cell stimulation induced by *Phytomonas tumefaciens* has been studied through attempts to change this property. Single-cell isolates have remained apparently unchanged in pathogenicity when kept for seven years in stock culture media. However, about 10 (sometimes more) culture generations in similar media containing 0.1 and 0.3 per cent glycine commonly attenuate these cultures. Glycine is relatively toxic and the smaller quantities are used until some tolerance is developed. The loss of pathogenicity is faster at pH 8.0 than at 6.0. A few cultures have regained pathogenicity in part or completely when restored to stock media. However, a number of them have remained attenuated for two years. Non-virulent strains appear similar to pathogenic sister cultures in colony characters, dye absorption, and reactions in differential media. The utilization of glucose and glutamic acid indicates that growth of the attenuated glycine acclimatized cultures is somewhat less than that of the sister pathogenic cultures. Of approximately fifteen related compounds only glycine, alanine, glycylglycine, and to some extent, dicyandiamid, cause loss of virulence.

Centrifugal Movement of Ceratostomella ulmi in Inoculated American Elms. S. L. SMUCKER. Young American elms held in a greenhouse under quarantine conditions were inoculated in incisions near the base of the stems in the spring of 1936. The trees were held through 2 growing seasons. No elm bark beetles or leaf-feeding insects were permitted to attack the trees at any time during the experiment. Seven to 70 days after inoculation 34 per cent of the trees developed active symptoms of disease. No trees were entirely killed the first season. The second growing season after inoculation 2.6 per cent of the trees that had active symptoms of disease the first year developed wilt and die-back. *Ceratostomella ulmi* was inoculated from a high percentage of all the inoculated trees from which cultures were made the second year. These preliminary results were obtained under controlled conditions in the greenhouse and no attempt is now made to extend an interpretation of them to field conditions.

A Fusarium Foot Rot of Cucurbita. WILLIAM C. SNYDER. A destructive foot rot has been observed in California on *Cucurbita pepo, C. moschata, C. maxima,* and certain varieties of these species. Italian Zucchini squash is particularly susceptible, but all of the many varieties of squashes, pumpkins, and gourds tested have succumbed. The disease occurs mostly in the coastal counties, and losses have ranged up to 75 per cent of the stand. There are indications that seed may serve as a carrier of the disease. Symptoms appear as yellowing and browning of the lower leaves and, finally, drooping of all leaves and wilting of the plant. The rot involves primarily the cortical tissues of the stem near the ground level but may proceed deeper and sever the plant completely from the roots. Fruits also may become infected. The causal fungus is a Fusarium belonging to Section Martiella. Single-spore cultures used in pathogenicity tests have proven the organism highly virulent. Susceptible plants grown in sterilized soil have developed symptoms within 2 weeks after watering the soil with a spore suspension of the fungus. Through manipulation of cultures it has been possible to obtain the perfect stage of the pathogen readily. This is found to fall in the genus *Hypomyces* of the Hypocreales.

The Epidemiology of Stem Rust of Wheat in Three Successive Contrasting Years. E. C. STAKMAN, W. T. BUTLER, R. U. COTTER, and J. J. CHRISTENSEN. Favorable weather permitted abundant uredial development in the North in the fall of 1934. Timely winds carried spores southward, and numerous infection centers developed, especially in early sown fields. Direct and circumstantial evidence indicated uredial overwintering in 8 per cent of wheat fields examined in Texas. Superabundant late-spring rainfall and delayed maturity of wheat in northern Texas and late wheat and "rust weather" in parts of Kansas and Nebraska favored abnormally heavy rust development. Favorable winds for northward dissemination of urediospores, late spring grain, ideal rust weather, and virulent physiologic races combined to cause a devastating epidemic in spring wheat. The uredial stage survived the 1935–36 winter in 2 per cent of Texas wheat fields examined. Spores were blown northward early, in May and June, but heat and drought prevented an epidemic. In 1936–37 uredial overwintering was negligible in Texas and northern Mexico, but inoculum was abundant farther south. Rust appeared and multiplied rapidly in northern Mexico, northern Texas, and beyond. Northward, repeated, heavy spore showers; rust weather; late, heavy wheat stands; and virulent rust races contributed to a wide-spread epidemic.

The Increase and Importance of Race 56 of Puccinia graminis tritici. E. C. STAKMAN and R. C. CASSELL. There have been pronounced recent shifts in population trends of several physiologic races, but the origin, extension in geographic distribution, and increase in prevalence of race 56 are most significant. Found first and only in Iowa, Kansas and Nebraska in 1928, it is now generally distributed throughout the United States and northern Mexico (in Canada also, according to reports of Dominion Rust Research Laboratory). It constituted 1 per cent or less of uredial isolates identified by the writers in the United States from 1928 to 1931, inclusive. The percentages since then follow: 1932, 2.1; 1933, 3.7; 1934, 33.1; 1935, 44; 1936, 47; 1937, 56 (to November 1). There is evidence that race 56 originated on barberry and that it was largely responsible for the collapse of Ceres wheat, moderately to highly resistant to stem rust prior to 1935. In 1937, race 56 constituted approximately 70 per cent of the isolates obtained from Ceres. That the aecial stage continues important in the origin and persistence of races is shown by the fact that from 1935 to 1937, inclusive, a different race was identified in every 6.5 aecial isolates, while the ratio for uredial isolates was 1:59. (U. S. Department of Agriculture and Minn. Agr. Exp. Sta.)

The Gymnosporangium Rusts in Maine. F. H. STEINMETZ and ALTON E. PRINCE. The Gymnosporangium rusts have been under observation since 1931. Their importance was accentuated by the occurrence of *Gymnosporangium clavipes* on certain varieties of apples. More recently these rusts have come to the attention of property owners as diseases of ornamental trees and shrubs. Eleven species of Gymnosporangium rusts have been collected in the aecial and telial stages on native host plants. They are here listed and numbered for referenc in this abstract. 1. *G. clavariaeforme*, 2. *G. clavipes*, 3. *G. aurantiacum*, 4. *G. davisii*, 5. *G. ellisii*, 6. *G. biseptatum*, 7. *G. transformans*, 8. *G. juniperi-virginianae*, 9. *G. globosum*, 10. *G. corniculans*, 11. *G. nidus-avis*. The telial stage of species 1, 2, 3, and 4 was collected on *Juniperus communis* var. *depressa*, a widely distributed host. The telial hosts of the remaining rusts are local in Maine. The same stage of species 5, 6, and 7 was collected on *Chamaecyparis thyoides*, while species 8, 9 and 11 were collected on *J. virginiana* var. *crebra*. Species 10 has been found on *J. horizontalis* only, while species 11 has been found on *J. horizontalis* and *J. virginiana* var. *crebra*. *G. clavipes* is widely distributed and is of greatest economic importance to apple growers in Maine.

The Copper Factor in Bordeaux Injury. R. F. SUIT and JAMES G. HORSFALL. From various experiments conducted to compare the efficiency of insoluble copper fungicides with that of Bordeaux mixture, it is indicated that the copper factor in Bordeaux is responsible for certain kinds of injury. From results obtained on apples, cucurbits, peaches, and Lima beans it is shown that the insoluble copper fungicides produce more leaf spotting than does Bordeaux. Further, in the case of apples, there is less fruit russet from Bordeaux. From data on cherries and tomatoes it is apparent that copper is responsible for some defloration. There are also results indicating that copper may accelerate transpiration. Copper injury of Lima beans is somewhat reduced by use of zinc dust, sulphite lye, or lime, and markedly so by magnesium oxide. Copper injury seems to be increased with dosage, solubility, and electrolytic dissociation of the compound and its tenacity to the plant. However, in various experiments, plants sprayed with Bordeaux have shown a stunting, deformation of leaves, reduced yield, or other symptoms not produced when Bordeaux substitutes were used. This would indicate certain symptoms are associated with Bordeaux injury that cannot be ascribed to the copper factor.

Observations on Nematodes of Buffalo Grass and Sorghum. GERTRUDE TENNYSON.

Effect of Mixed Inocula on the Production of Seedling Blight of Flax. IAN W. TERVET. Investigations on the possibility that weakly parasitic strains of *Fusarium lini* were mainly secondary organisms following upon the attack of fungi known to cause rotting in flax roots yielded negative results. Flax seedlings, grown in steamed soil in which cultures of *F. lini* had been mixed with isolates of several root-rotting fungi (*Helminthosporium* spp., *Rhizoctonia solani, Ophiobolus cariceti, Thielavia basicola*), showed a delayed emergence and a retardation of wilting. However, the attack of a very virulent strain of *F. lini* was completely changed by the presence in the soil of *Chaetomium* sp. Seedlings of Winona flax normally were killed by this strain of *F. lini* within one week after emergence, but, after 24 days in the soil with mixed inoculum, many plants were still alive. The root systems of the surviving plants were malformed, with short swollen lateral roots somewhat resembling the mycorrhiza of *Pinus*, while the stem internodes were very short and the foliage dark green. One-third of the plants survived until flowering time, although they were extremely dwarfed.

Further Work on Purification of Tobacco Mosaic Virus. CARL G. VINSON.

Soil Temperature in Relation to Potato Yellow Dwarf. J. C. WALKER and R. H. LARSON. Epidemics of yellow dwarf in central Wisconsin have been characterized by poor stands rather than high percentages of plants showing systemic symptoms. The major crop often is exposed to high temperatures in the light, sandy soil during and immediately following planting in the first half of June. Experiments were conducted in soil held at constant soil temperatures of 16°, 20°, 24°, and 28° C., and a common air temperature of 20–22° C. Infected seed tubers were cut into 4 pieces and one piece planted at each temperature. At 16°, normal germination was followed by development of plants indistinguishable from the control. At 20°, emergence was normal but plants were slightly dwarfed. At 24°, emergence was reduced and severe symptoms occurred. At 28°, there was almost no emergence. Seed pieces in nonmerging hills usually remain firm for several weeks, often until harvest. Even after that period, they commonly germinate normally in cool soil.

Present Status of Azalea Flower Spot. FREEMAN WEISS and FLOYD F. SMITH. This disease, caused by *Ovulinia azaleae* n.g. et n.sp. occurs throughout the South Atlantic and Gulf Coast region from Wilmington, N. C., to central Louisiana. It chiefly affects cultivated azaleas of the Indian and Kurume groups. Native azaleas, though susceptible, have not been found infected in nature. Environmental requirements for infection parallel the conditions favorable for host blooming and are approximately satisfied in both greenhouse and outdoor azalea culture whether in the South or North. The pathogen is disseminated by the transportation of infected blooming plants and also, apparently in the soil, with non-blooming plants. Its survival between flowering seasons outside its present range has not been demonstrated. Several native plants besides azaleas, including rhododendrons, *Kalmia*, and *Vaccinium* spp., are potential hosts. Satisfactory control under nursery conditions was obtained by semi-weekly applications of copper-kaolin dust throughout the blooming season, but this is not a practicable control in show gardens. Insects were apparently not responsible for initiating primary infections on azaleas, but became efficient carriers during the height of bloom, and can be important factors in spreading the disease throughout a planting, or introducing it into nearby plantings. Abrasion of flowers by bees does not promote infection. Insects most important in dissemination of flower spot are *Apis, Bombus, Emphoropsis* and *Xylocopa*.

Effect of Phytomonas flaccumfaciens, Phyt. insidiosa, Phyt. michiganensis, Phyt. campestris, Phyt, panici, and Phyt. striafaciens on Maize. E. J. WELLHAUSEN. Effect of above organisms was determined on 2 inbred lines of maize, one (GB797) very susceptible and the other (OSF) very resistant to *Phyt. stewarti*. Infection of very young seedlings of these 2 lines was obtained with each of the organisms. Each seemed to attack GB797 more readily, with the exception of *Phyt. insidiosa*, which caused more infection on OSF. Infection of either host was primarily confined to the xylem vessels of the vascular bundles. No brown, water-soaked lesions, characteristic of infection with *Phyt. stewarti*, were produced. *Phyt. flaccumfaciens* usually caused a high degree of infection on GB797. Seedlings were generally severely dwarfed and often a few were killed. *Phyt. insidiosa* had a similar effect on OSF. Infection of the 2 maize lines by the other organisms was much milder. *Phyt. campestris* and *Phyt. striafaciens* had practically no effect on OSF. *Phyt. stefarti* was tried on the common hosts of some of the organisms tested on maize. Golden Cluster beans were slightly infected. Proso millet and Early Pearl

oats were readily attacked. Noso infection on Bonny Best tomatoes or Early Jersey Wakefield cabbage was detected.

Early Blight of Tomato in Wisconsin. O. C. WHIPPLE.

Two Strains of Cucumber Virus on Pea and Bean. O. C. WHIPPLE and J. C. WALKER. One virus (408) was recovered in 1935 at Madison, Wisconsin, from naturally affected peas and beans growing in the open, while the other (729) was recovered from peas in a commercial greenhouse. Strain 408 produced symptoms on tobacco and cucumber similar to *cucumber virus 1;* 729 produced a mild mottle on tobacco and symptoms on cucumber similar to *celery virus 1.* By transfer of expressed juice from tobacco infected with 408, symptoms resulted on 21 species in 5 families, including 4 varieties of *Pisum sativum,* and 39 varieties of *Phaseolus vulgaris* including some of the latter resistant to *bean virus 1.* All hosts of *cucumber virus 1* tested were susceptible. Mottling of leaves of young plants and mild streaking of stem were produced on pea. Distinct mottling, leaf distortion, and stunting occurred on bean and, with one substrain, a severe streak. Strain 729 caused leaf necrosis on pea, but no symptoms on bean. Its host range closely parallels that of *cucumber virus 1.* Both 408 and 729 are transmitted by *Myzus persicae* and are similar to *cucumber virus 1* in their point of inactivation through ageing *in vitro,* dilution, and heating.

Further Tests of Rosin-lime Sulphur as a Fungicide. C. E. YARWOOD. In 1937 field tests of fungicidal control of snapdragon rust, in which rosin-lime sulphur, lime sulphur + spreader, sulphur dust, cottonseed oil, and lime sulphur + cottonseed oil were employed, 1 per cent rosin soap + 1 per cent lime sulphur was the most effective spray, reducing the rust infection from 44 pustules per leaf on the checks to less than 4 per leaf on the treated plants (4 check and 4 treated plots) and increasing the yield from 620 mg. per plant on the check to 1620 mg. per plant on the treated plants. In field tests of fungicides for the control of onion downy mildew, rosin-lime sulphur was superior to Bordeaux + spreader, lime sulphur + spreader, or Palustrex copper compound. In the greenhouse, rosin-lime sulphur, applied to the lower surface of bean leaves, greatly reduced the mildew infection on the upper surface of the same leaves.

The Effect of Boron Nutrition on the Susceptibility of Some Plants to Powdery Mildews. C. E. YARWOOD. Under conditions of heavy artificial inoculation, check sunflower plants in Hoagland solution minus boron were severely stunted and heavily mildewed, while plants with 1 and 10 parts per million of boric acid made a normal growth and were much less mildewed in most tests. The addition of boric acid, up to toxic quantities to sunflowers in greenhouse soil not deficient in boron, had no specific effect on the mildew susceptibility of the plants. The addition of boric acid to water cultures of beans, cucumbers, and oats, and to soil cultures of beans had no marked effect on the mildew susceptibility of the plants.

Some Diseases in Forest Plantings of White and Red Pine in Western New York. HARLAN H. YORK. These plantings have been established for 20 to 30 years. Until 1929, when disease first became evident, they were fully stocked. The plantings consist of a single species and the rate of growth of the trees is above the average for each species. The determination of the more important predisposing factors is being attempted. The damage from *Polyporus schweinitzii* and a resinosis is so severe that it is doubtful whether the trees in plantings having a total area of over 1000 acres will reach maturity. These disturbances are most destructive in soils of high pH and colloidal content and where there is a slow or poor humification of the duff. *Polyporus schweinitzii,* if not distributed from nurseries, may attack trees within a very few years after the plantings are established. It has been isolated from the roots of northern white-pine trees that had been in the plantings 7 years.

Weighted Percentages of Resistance of Tomato Varieties to Fusarium lycopersici. P. A. YOUNG and J. J. TAUBENHAUS. Wilt resistance of tomatoes was tested extensively in 3 fields under epiphytotic conditions. Weighted percentages of economic resistance of tomato varieties to *Fusarium lycopersici* were calculated on the basis of time and severity of appearance of wilt symptoms. Tomato varieties are recorded in the numerical order of their percentages of wilt resistance, beginning with the least resistant. Varieties 19 to 44 per cent resistant were: Stone, Columbia (Haven), Norton, Greater Baltimore, Nystate, and Tomato 1111. Varieties 45 to 69 per cent resistant were: Gulf State Market, Globe, Marvelosa (Vaughan), Gulf State 20–5, Clarks Special B, Break O'Day Urbana Forcing, Lloyd Forcing, Marvelosa (Porte), Early Baltimore, Pritchard, Louisiana Pink ×

Walter Richard, Grothens Red Globe, Marvana (Haven), Long Calyx Forcing, Illinois Pride, Columbia (Porte), Prairiana, Kanora (Barteldes), and Marhio. Varieties 70 to 91 per cent resistant were: Sureset Forcing, Michigan State, Marglobe, Rutgers, Guba's Crosses 421 and 424, Marvel, Blair Forcing, Louisiana Red, Kanora (Melchers), Buckeye State, Marvana (Porte), Marglobe 75, Norduke, Louisiana Pink, and Marglobe 63. Twelve selections of Gulf State Market tomatoes were 34 to 64 per cent resistant. X-ray treatment increased resistance of Marglobe in 1936.

Further Work on Purification of Tobacco Mosaic Virus. CARL G. VINSON. Active virus material in quantity has been obtained by others by employing ammonium sulphate in salting out the virus. The use of a nitrogenous salt in salting out the virus is obviously undesirable. The virus fraction may be precipitated from large quantities of juice from diseased plants by careful acidification. The precipitated virus fraction is readily redispersed by suspending in M/3 phosphate solution of about pH 7.0. Solid, anhydrous sodium sulphate may be used to force the virus out from the phosphate solution in definite crystalline form. Dilute acetic acid may be used to wash the crystals almost free of ash. Shaking the crystalline product with diethyl ether concentrates the pigment on the films. Thus, a practically snow white product is obtained.

Apple-tree Measles. H. C. YOUNG and H. F. WINTER. Measles, as here considered, relates primarily to the pimply and necrotic condition of the inner bark typical of Red Delicious apple trees. After several years' study it was concluded that the disease is non-parasitic. The large number of isolations remained sterile. Grafting, injections of juice from diseased twigs, and budding, using buds from diseased trees, all, failed to produce the disease. Mineral-deficiency studies were next tried. Using Shive's complete nutrient R_3S_3 at one-half atmospheric concentration in purified sand cultures, 7 series of 4 Red Delicious trees, each, were grown for 2 seasons. Series 1 was fed the nutrient solution and the remaining series were given, in addition, 1 and 2 p.p.m. boron, zinc, and manganese, respectively. During the first season fairly normal growth occurred in series 1, whereas the boron-fed trees made about twice the normal growth. A slight toxicity occurred in the zinc and manganese series. After 30 days of the second season, pimples began to appear generally except where boron was used. As the season advanced the inner bark of the trees showed a typical necrotic condition of measles, as it appears in the orchard. Pimply trees, fed on boron, cleared up.

GALL DEVELOPMENT ON PINUS SYLVESTRIS ATTACKED BY THE WOODGATE PERIDERMIUM, AND MORPHOLOGY OF THE PARASITE[1]

R. P. TRUE[2]

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INTRODUCTION

A *Peridermium* attacks Scotch pine, *Pinus sylvestris* L., plantations bordering Round Lake near Woodgate, New York, causing susceptible trees to form globoid galls. Since its discovery there by York (13) in 1925, it has been called the Woodgate rust or the Woodgate *Peridermium.* York's observations led him to believe that the rust has been present since 1895 in these plantations, some of which, planted in 1870, are among the oldest in America. Some trees of *P. sylvestris* do not form galls after natural infection or inoculation with the fungus. Hutchinson (6) has recently investigated this problem of resistance. The present study deals with the reactions of susceptible trees and the morphology of the rust fungus.

INOCULATIONS AND FIELD NOTES

One hundred forty field inoculations were made upon 27 trees of *Pinus sylvestris,* ranging in age from 9 to 30 years and located in the infection area, supplemented the naturally infected material available for study. The inoculated trees varied widely in susceptibility, as indicated by the varying number of galls they bore from natural infection. The inoculations were made between the 7th and 30th of June from 1930 through 1933. Susceptibility was found to be highest during the first half of June and to decline toward the end of the month.

The aecial inoculum was obtained from fruiting galls collected in the vicinity. Spores for immediate use were carefully removed from the galls as soon as the latter were collected. Galls whose spores were to be used in later inoculations were placed indoors in a dry shaded place and the viability of the spores was tested periodically by placing samples of the spores on fresh well water and noting their percentage germination. Inside storage was resorted to because the spores of galls left late in the field often are blown away or washed out, or they germinate in the aecium.

The method of inoculation was that employed by York,[3] using the cellu-

[1] A dissertation submitted to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] The writer is indebted to Dr. H. H. York for his advice and criticism during the progress of this work, and to Dr. Conway Zirkle and Dr. K. D. Doak for many helpful suggestions. He also wishes to acknowledge the aid of the Division of Forest Pathology of the Bureau of Plant Industry of the United States Department of Agriculture. The photographs were made with the help of Mr. Vernon Doney with the exception of figure 1, C, which is from one of Dr. H. H. York's negatives.

[3] York, H. H. Celluloid "iceless refrigerator" and some results from field inoculations with the aeciospores of Woodgate rust (*Peridermium* sp.). [Manuscript.]

loid iceless refrigerator. Early inoculations were made upon the stems of the current season's shoots at the time when their elongation separated the developing needles sufficiently to permit a direct inoculation upon the surface of the stem. The needles borne on the young shoots had elongated to more than half their mature size before the last inoculations were made.

Field observations and collections of artificially and naturally infected materials were made each year. During the summer of 1933, observations were recorded and collections made daily for periods of from 3 to 7 days after inoculation and thereafter at weekly intervals. In other years, after the month of June, the intervals between collections varied from 1 to 2 months until dormancy. Notes and collections also were made in late November and in May to reveal the developmental stages present shortly after entry of the host into the winter condition and its breaking dormancy.

ANATOMICAL STUDIES

Methods

Newly inoculated shoots, developmental stages, and mature galls were fixed in the field. Bouin's solution, formal-acetic-alcohol, and formalin-alcohol proved the most satisfactory of the 13 fixatives tried. The first and the last gave the best fixations of the fungus. During fixation, air was removed from the tissues by placing the vials containing the specimens in a closed chamber and evacuating it with a suction pump.

After 12 to 48, and, in some cases, 72 hours of fixation, most of the material was dehydrated and transferred into pure hard paraffin by Zirkle's (14) butyl-alcohol method. Tissues not imbedded at once were stored in the dehydrating mixture made up of 30 parts of water and 50 to 20 parts of ethyl and butyl alcohol, respectively, where they remained reasonably soft until dehydration could be completed.

Early infection and developmental stages for histological study as well as three-year old galls were cut from 8 to 15 μ thick. Material with sori in the bark was cut as thin as 3 μ. Land's method, using a saturated solution of gum arabic followed by a 1 per cent potassium dichromate solution to float out the ribbons, was found most satisfactory in fixing the sections to the slide.

The stains that most clearly differentiated the mycelium in its relation to the host tissue were Orseillin BB in a 3 per cent aqueous solution of acetic acid, counterstained with a similarly acidulated aqueous solution of anilin blue, or with a saturated solution of crystal violet dissolved in clove oil. By following a procedure recommended by Doak,[4] crystal violet in clove oil was used alone to show excellent detail in hyphae and haustoria, but it often necessitated destaining the host tissues too far to show them clearly. The combination of Heidenhain's haematoxylin and iron alum was used to show nuclear phenomena and, in combination with safranin, to show the histological details of the gall.

[4] Personal communication.

For field study, the following rapid method was used to obtain permanent slides: Fresh material was cut in water on a portable sliding microtome and placed at once in Bouin's fixative, where it remained for 10 minutes. It was then washed in water until no further color of the fixative came from it and set to stain for five minutes in Orseillin BB. Excess stain was washed out in the early steps of dehydration and, after a brief clearing, in clove oil followed by xylol, the material was mounted in balsam. The whole schedule may be completed in from 30 to 45 minutes.

External Morphology of Gall Development

In order to make it easier to follow the sequence of internal events described under histological studies, it seems desirable to give a brief summary of the external phenomena that often follow infection and accompany gall development, as observed in inoculated material and supplemented by observations of galls resulting from natural infection. While the phenomena here considered often occur as described, gall formation is very variable and the times and sequence of events show such variation that this brief outline must be considered only as an aid to the understanding of the histological phenomena involved.

Infection occurs primarily through the epidermis of the current season's shoots. Points of infection often are marked by spots that usually range from orange to dark brown. These sometimes appear in the first week of July. Their margins are often sharply defined, but frequently are less definite, and may appear water-soaked. Infection spots do not necessarily indicate successful invasions. Infections whose spots show indefinite or water-soaked margins seem more apt to produce galls than those with more sharply delimited spots. As observed, slight swellings may result in the season of infection below many infection spots and spot-free points of infection. In the second season, dead shrunken cortical areas may appear in the central portion of the surfaces of the swellings, which by the end of that season may become distinct spherical or hemispherical galls. In May of the third season, two years after infection, slight colorless exudates are at times found near the margins of the sunken areas, which, in some cases, may be associated with internal pycnia. Toward the end of the third season, the central sunken area occupies most of the gall surface, and is bounded by a slightly raised rim.

During the fourth season the gall usually acquires those external characteristics which typify it during its further enlargement (Fig. 1, A). Aecia are produced in the central sunken areas of the roughly spherical or hemispherical galls (Fig. 1, D). As the aecia develop, they push up the dead tissues, which usually scale off, eventually exposing the often confluent fruiting surface (Fig. 1, C). Since the layer of aecia-bearing tissue is in turn usually exfoliated before winter (Fig. 1, E), the central portion becomes more and more sunken in comparison with the peripheral rim, which then appears as a collar delimiting the gall (Fig. 1, A, C, and E). On an occa-

sional tree, the periderms of the galls are not sloughed off but, as swelling continues, are cleft by deep cracks in which the aecia may be borne. (Fig. 1, F.)

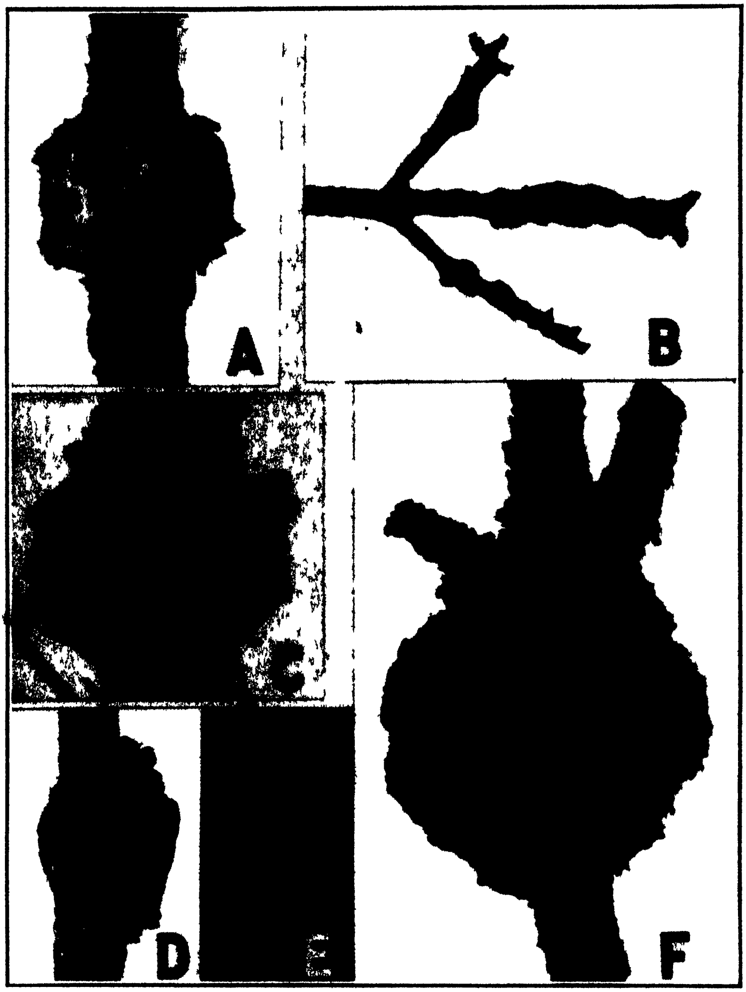

FIG. 1. A. Typical spherical gall with sporulation well advanced. B. Young fusiform swellings due to multiple infections. C. Gall with sporulation completed. Note complete exfoliation of periderm external to aecial layer. D. Hemispherical gall. E. Gall with aecial layer being sloughed away by a new periderm formed benath. F. Ribbed gall with adhering periderm.

Galls on an occasional tree become necrotic and frequently develop abortively, or are misshapen, often through necrosis originating in their central areas, which then appear much more deep-sunken than in normal galls. Such necroses may well at times be caused by invading secondary fungi. Mass infections resulting from inoculations of twigs of highly susceptible trees may cause an extensive killing of the outer layers, which, with subsequent swelling, crack deeply into the woody tissues forming longitudinally

confluent furrowed swellings. These sometimes fail to produce galls or develop into roughly fusiform compound galls (Fig. 1, B), which fruit very sparsely, if at all.

Histology of Invasion and Host Reaction

Epidermis and Primary Cortex. Most successful infection occurs preceding periderm formation. During this period the stem is protected by an epidermis whose outer walls are, in general, heavily lignified and are covered with a thick cuticle (Pl. I, 1). At the bases of needle fascicles, however, walls of the epidermal cells are uniformly thick, apparently uniformly lignified throughout, and usually are pitted. At the fascicle bases, the outer walls of even the subepidermal cell layer become lignified and sunken stomata appear. The cells of the first subepidermal layer are large and radially elongate (Pl. I, 1). Other nonspecialized cells of the cortex appear to be typical of parenchyma tissues in general. Cells of the resin ducts found in the inner cortex are vertically elongate and often contain tannin-like substances, as do a few lignified cells with pitted walls, which are scattered through the cortical parenchyma. About the middle of June a meristem arises from the subepidermal cells or from the cell layers beneath, forming 2 or 3 rows of thin-wall, tangentially elongate cells, the phellogen, external to which the cells die and gradually collapse (Pl. III, 7).

All infections, observed histologically, have been made through the epidermis, either inter- or, more frequently, intracellularly, in the less lignified portions. Appressoria have not been noted. (Fig. 3, D). The germ tube penetrating the cuticle and outer walls of the epidermal cells is considerably narrowed, but, once inside, it broadens, forming a vesicle, and may branch before leaving the cell to enter the subepidermal intercellular space (Fig. 3, A). In the cases of cells penetrated by germ tubes, the thickening of the outer walls was at times much less pronounced than in unpenetrated neighboring epidermal cells. This seems not to be a matter of selection by the fungus, since no unpenetrated walls of comparable thinness were found in the same or similar material. If it is a result of fungus invasion, whether of actual dissolution of the wall or of preventing its normal thickening, it is produced quickly, since it is already obvious in material only 16 days after inoculation. Once the infecting hypha passes from the epidermal cell into the intercellular space below, it branches readily and sends hyphae into neighboring epidermal and subepidermal cells. These hyphae appear to function as haustoria, but, unlike the typical haustoria formed deeper in the cortex, they are often septate and may even branch (Fig. 3, B). This tendency toward intracellular development is short-lived, and the mycelium soon establishes itself intercellularly with typical haustoria.

While trees upon which galls develop are here considered susceptible, a large percentage of the infections fail to result in gall formation, even on the most susceptible trees. Histological studies of early stages of the invasion of such trees have shown that, while some infections meet with no apparent

FIG. 2. A. An invasion resisted partially by cell hypertrophy as at A. B. An invasion spreading vertically along the resin canal (A) has stimulated the adjacent tissues to cell division and radial cell orientation accompanied by marked reduction of intercellular space. C. An invasion being effectively walled out by a cicatrizing zone. D. Radial longitudinal section of infected phloem and xylem. Note broad rays with abundant intercellular hyphae (A); short, blunt irregularly pitted tracheids (B); abundance of phloem parenchyma (C); and resin-containing cavity (D) associated with radial resin canal not shown in this section. E. Transverse section showing wedge-shape area of gall wood with adnormalities intensified at the edge. Note also the internal and external areas of killing in the cortex. F. Longitudinal section showing a late stage in the development of the "collar" with living tissues considerably isolated radially by the death of cells interior to them.

resistance, others are constantly opposed by partial barriers, and yet others may at once be surrounded by barrier zones and walled out completely. In the course of the present study, a shoot no more than 2 cm. long, sectioned serially a month after inoculation, was found to have 24 infections. Five of these showed no trace of recognizable mycelium among the very abnormal

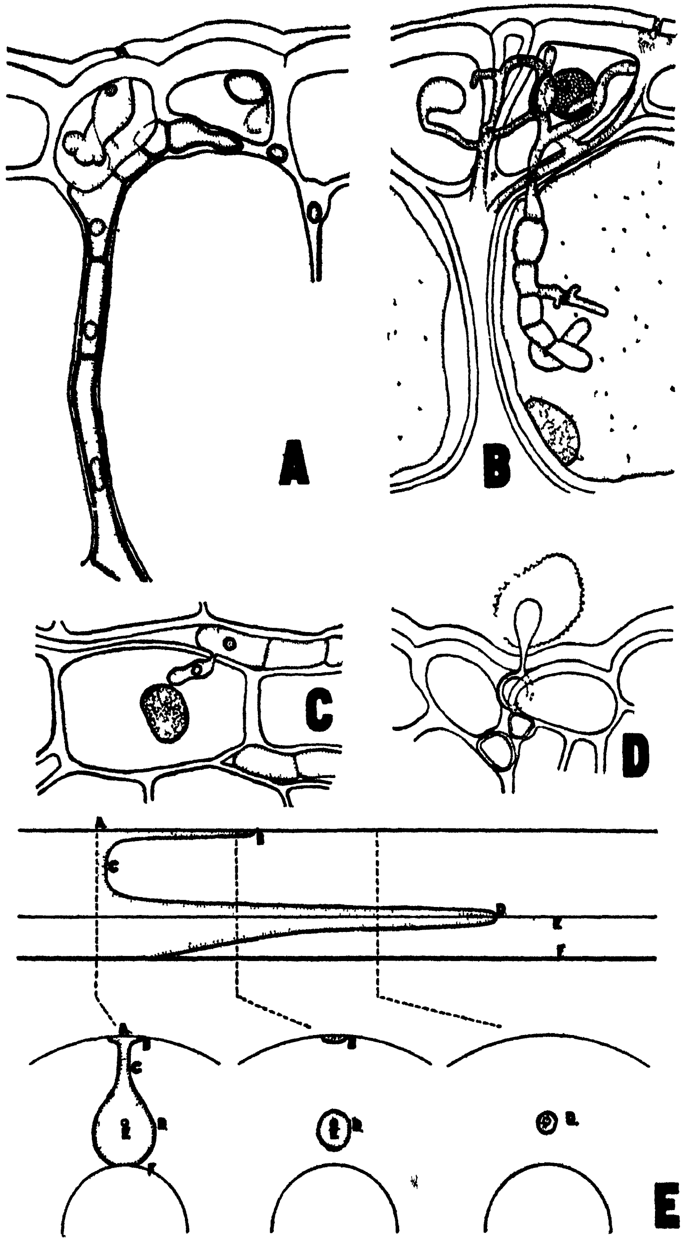

FIG. 3. A. Usual intracellular type of penetration showing vesicle in epidermal cell and subsequent intercellular development. B. Branching septate haustorium within a subepidermal cell. C. Mycelium and haustorium in the phloem parenchyma. D. Spore with short germ tube entering host, showing absence of appressorium and the intercellular type of penetration. E. Diagrammatic representation of the spread of the mycelium of a typical unresisted infection through the primary cortex. Radial longitudinal spread is shown and the appearance of transverse sections taken at various levels is indicated: A, point of penetration; B, extent of subepidermal spread; C, path of radial growth; D, extent of spread adjacent to resin duct in inner cortex; E, cortical resin duct; F, the phloem.

and often dying cortical cells. Most of them showed isolated groups of living hyphae penetrating the more normal portions of the cortex and the intercellular remains of others where tissue abnormalities were more severe. In the case of one infection, discovered in sectioning, where no external symptom was present, the abundant and continuous spread of the large intercellular mycelium had produced almost no abnormality in the areas it penetrated. This infection had penetrated as deeply into the cortex as most of the others, some of which were still confined to the outer cortex.

Infections to which little or no resistance apparently is offered are those that cause but slight early abnormality in the host. The abundant large hyphae pass between or encircle nearly every cell in the infected area (Pl. I, 2 and 3), and many cells are penetrated by the ellipsoid to cylindric haustoria, which are uninucleate and greatly attenuated where they pass through the cell wall (Fig. 3, C and Fig. 6, E). The haustoria often appear to seek out the nucleus of the host cell and those touching it appear at times to be flattened at the point of contact. The host cells in the infected zone are more spherical and regular in outline than those in the normal cortex, and there is a tendency toward hypertrophy, which is most pronounced in the cells invaded by haustoria.

The hyphae of a mycelium whose invasion apparently is unresisted advance in characteristically different directions (Fig. 3, E). Some run parallel to the axis of the twig in the spaces between the first one or two cortical cell layers or just beneath the epidermis. The major portion of the mycelium, however, spreads radially inward through the outer cortex until the inner cortex is reached, and there a second vertical spread occurs, which is most pronounced in the intercellular spaces adjacent to and near the epithelial cells of the resin ducts. These cells often show a tendency to hypertrophy, increasing in size until they may partially or completely fill the ducts. (Pl. I, 2). The mycelium often spreads a considerable distance about the margin of the phloem before entering it. (Pl. I, 3).

Fresh shoots of the current season's growth, collected September 20, 1933, and tested microchemically, indicated a possible physiological explanation for the diversified paths followed by the mycelium in unresisted infections. The layer of cells just below the phellogen, and at times the cells of the phellogen itself, well established at that date, were found containing starch, particularly near the bases of the needle fascicles, where every cell was filled with it. Elsewhere in these peripheral tissues starch was less abundant, appearing only in scattered cells. Internal to this layer the cells of the outer cortex seldom showed starch except below needle fascicles where starch-containing cells are few and scattered. The inner primary cortex, however, had numerous scattered starch-containing cells in the vicinity of the phloem and near the resin ducts. Droplets of fatty substances were present in scattered cortical cells, but were especially abundant in the cells surrounding the resin ducts. Compounds giving a positive reaction to the ferric chloride test for tannins were found in scattered cells in the inner cortex, but especially in the

cells bordering the resin ducts, where they appeared as coarsely to finely granular and, at times, even as amorphous material.

The presence of these substances, at least two of which may be associated with nutrition, in those tissues where the growth of the mycelium is most rapid, suggests that the localization of foodstuffs within the host may influence the paths of invasion in the cases of unresisted infections.

Partially resisted invasions tend to follow the same paths as those whose progress is unopposed, but they meet with localized mechanical resistance from the host, which often alters the direction and curtails the extent of their spread.

Three mechancial responses offer some degree of resistance to the invading mycelium. The first is exaggerated hypertrophy of the cells invaded and often a less pronounced enlargement of contiguous cells (Fig. 2, A). This diminishes the space between the cells, opposing the intercellular proliferation of the mycelium. Close-pressed hyphae in varying stages of abnormality have been observed wedged between the walls of enlarged cells in areas of severe cell hypertrophy. In other cases the advancing hyphal tips may grow beyond the enlarging cells before the extreme stages of hypertrophy are attained and so escape this means of mechanical resistance. The difference in degree of hypertrophy in the invaded host cells seems to be one measure of the compatibility of the reacting organisms.

A second structural response usually offers some resistance to invasion and, like excessive cell enlargement, seems to indicate considerable incompatibility between the host and the invading mycelium. Mature cortical cells in and near the infection area divide, filling the space they formerly occupied with a larger number of smaller cells often oriented in rows radiating from the infected area (Fig. 2, B). The radial arrangement of the cells might appear to favor a swift invasion, but it usually is accompanied by the almost complete elimination of intercellular space, which again appears to be the morphological barrier to further invasion. Both hypertrophic and hyperplastic reactions appear to be direct responses to the invading fungus.

The host often responds structurally to invasion in a third way. A row of phellogen cells derived from mature elements of the primary cortex produces a distinct zone of cicatrization next to infections in which host cells have been killed. This layer cuts off the dead cells, together with some of the very abnormal cells adjacent to them, from the more normal tissues. This cicatrix often serves as a successful barrier against further invasion but does so only when the host cells attacked die so swiftly that the cicatrizing zone walls out the invading mycelium before it has had time to spread (Fig. 2, C). In case of an infection by a mycelium fairly compatible with the host, invaded areas remain nearly normal for some time after invasion during which the invading mycelium spreads deep into the host tissue before any of the invaded cells die, stimulating cicatrization (Pl. II, 2).

Microchemical tests made upon material showing partially resisted inva-

sions revealed that abnormalities in the visible cell contents appear in tissues before they show the structural deviations described above. Starch is unusually abundant in the early stages of physiological abnormality. It is present in large quantities in the morphologically normal cells at the margin of the infection, both before and after the tissue has been invaded and its cells penetrated by haustoria. The presence of these large deposits of starch within and adjacent to the infected areas is accompanied by a scarcity of it elsewhere in tissues in which it normally occurs. Fatty globular substances appear abnormally abundant in the pathic living cells and seem abnormally scarce in adjacent uninvaded tissues. Tannin or tannin-like substances are universally found in cells that have passed the early stages of abnormality; and their appearance often is synchronized with the complete disappearance of starch and fatty substances from the pathic cells. They appear in the wall or lumen of nearly every dead cell.

In the case of barrier formation, the thin-wall cells of the barrier are not cutinized nor lignified, nor do they contain starch, fatty substances, tannin, or tannin-like compounds. They give a negative reaction to tests with Scarlet R; but Hutchinson (6), using ammoniacal gentian violet as a reagent, found them to be suberized in the material with which he worked. A globular fatty metabolite, staining pink with Scarlet R, is often present in the meristematic cells below the barrier, and scattered cells of the adjacent cortex, and starch grains appear similarly distributed.

Whether the invasion is resisted or not, invaded cortical tissues eventually die. Their death occurs generally in the same chronological order as their invasion, so that, in cases of at least moderate compatibility, an external area of killing appears close beneath the epidermis to correspond with the early vertical spread of the mycelium there, and a similar but more extensive internal area is located in the inner cortex, where the internal vertical proliferation took place. These are united opposite the point of epidermal penetration by a smaller area of killing in the outer cortex, where the centripetal spread occurred. (Fig. 3, E).

External areas of killing usually involve the epidermal cells, as well as those directly interior to them, and are clearly visible to the naked eye as infection spots. When these spots are minute or have sharply defined margins, it may be taken as an indication that the invasion is being at least partially opposed by the host; particularly, if they appear within a month or six weeks after inoculation. Larger spots having indefinite margins often appearing water-soaked are likely to mark successful infections, and especially so if their appearance is considerably delayed. In cases of extreme compatibility, swellings may appear before their infection spots. Most infection spots on susceptible trees at Woodgate show intermediate characters.

Peripheral and longitudinal spread of the fungus is limited, at first, almost entirely to the primary cortex, and, later, to the phloem. The mycelium spreads more swiftly in the inner than in the outer cortex, and the death of the invaded cells of the former cuts off the partially invaded and

mostly living cells of the latter from radially conducted water and nutrients. (Pl. I, 4). Thus isolated, they in turn die and collapse against the dead inner cortex, forming a sunken area at the surface of the central portion of the swelling. The peripheral areas of the outer cortex, only partially, or not at all so isolated, remain alive for some time as a rim or collar. This becomes more pronounced after the middle or the end of the second season when the infected cambium usually has cut off considerable phloem tissue, which serves thereafter in the place of the cortex as the chief region of vertical and peripheral spread of the mycelium. In the third season, on fruiting, the dead tissues of the central sunken area are sloughed away, making the collar even more prominent (Fig. 2, F). In the enlarging gall this collar gradually dies back as its edges are further isolated by the continual dying of the tissues internal to it, but it remains distinctly prominent in the case of most mature galls, even after several years.

While responses of resistance often fail to prevent gall formation on susceptible trees, they may so limit peripheral spread of the organism that a hemispherical rather than a spherical gall is produced (Fig. 1, D), or they may hold the mycelium in the cortical region for one or more seasons, thus delaying gall formation. The mycelium of an unresisted infection was noted penetrating the cambium on July 20 of the season of infection and, by August 1, the hyphae of several of that season's infections, some of which had been resisted, had penetrated beyond the cambium. One case was noted in which a resisted infection was just entering the phloem on June 15 of the season following that of its epidermal penetration.

Matured Stelar Tissues. The mycelium passes through the intercellular spaces of stelar tissue derived from uninvaded cambium largely in a radial direction. The structural response of the cells of phloem tissue to the presence of the fungus is limited mostly to cell enlargement, but a division of some of the matured cells at right angles to the line of invasion, and even the formation of a barrier, have been observed in exceptional cases. Penetrating mostly along the phloem rays, the mycelium crosses the cambium at right angles and continues its radial path mostly along the medullary rays for but a short distance into the xylem, where it fails to cause any appreciable structural abnormalities.

Like the cortex, however, the stelar tissues may undergo a marked physiologic response because of the presence or even the proximity of the fungus. Starch grains and fatty droplets, present to some degree in the normal parenchymatous cells of both phloem and xylem, are often present there in abnormally large amounts in tissues adjacent to, as well as within, infected areas.

Cambium. While the fungus usually causes but slight structural abnormality in the matured phloem and apparently none in the xylem, its presence stimulates the cambium to the production of definite and characteristic abnormalities in the phloem and xylem formed after cambial invasion. In view of Bailey's (3) work, indicating that the length and volume of tracheids of normal gymnosperms are closely correlated with the length and volume

of their cambial initials, it seems likely that these abnormalities reflect abnormalities in the cambial initials induced by the presence of the fungus in the cambium.

The cambium usually is first penetrated at a point or over a limited area adjacent to the point of infection, and subsequent cambium invasion comes always from the phloem, never from vertical or peripheral spread of the mycelium within the cambium itself (Pl. II, 4 and 5, and Pl. III, 6). Since the cambium is stimulated rather than killed by the invasion, normal and invaded portions are found functioning side by side and producing normal and abnormal phloem and xylem adjacent to each other (Pl. II, 1). Newly invaded cambium, *i.e.*, that at the edges of the invaded areas, produces phloem and xylem cells showing greater abnormality than those at the center of the areas that have been invaded for some time. This fact, together with the relatively slow rate of invasion of the adjacent normal cambium areas, often results in the formation of a more or less wedge-shape body of gall wood whose edges show greater abnormalities than its center (Fig. 2, E).

Among the abnormalities found in tissues derived from invaded cambium are the following:

1. Increase in radial and tangential diameter of both phloem and xylem elements. In the case of the xylem this often is accompanied by a pronounced shortening of the tracheids, which are often blunt (Fig. 2, D, Pl. II, 4 and Pl. III, 6).

2. An 80 to 100 per cent increase may be shown in the number of xylem cells formed. No comparable increase is shown in the number of phloem elements.

3. Increase in the proportion and number of parenchymatous cells in both phloem and xylem. In the phloem these almost completely replace the sieve tubes (Pl. II, 1), while, in the xylem, the number of parenchymatous cells associated with the resin ducts is so increased that often continuous areas of parenchymatous cells extend from one duct to another, particularly in the areas at the edges of the abnormal wood.

4. Increase in the number of vertical resin ducts in the xylem. This increase, however, is roughly in proportion to the increase in the volume of gall wood, and, though their diameters are abnormally large, many of them are partly or entirely closed through the swelling of their epithelial cells (Pl. II, 5).

5. Frequent production of radial resin passages in the fusiform rays of the phloem, which terminate next to the cortex in resin-containing cavities, somewhat spherical as seen in transverse and in radial longitudinal sections. These have been observed very seldom in normal wood (Fig. 2, D, and Pl. II, 3).

6. Increase in the number, height, width, and cell volume of linear rays in both phloem and xylem. (Fig. 2, D; Pl. III, 4 and 5). The number of xylem rays may be increased by 50 to 60 per cent, as seen in cross section. While the height of normal xylem rays is from 1 to 5 cells and the rays are

comparatively widely separated vertically, abnormal rays vary from 2 to 6 cells high and often appear almost vertically confluent one with another as seen in tangential longitudinal sections (Pl. III, 4 and 5). Width of normal linear rays of the xylem is 1 and very exceptionally 2 cells, while those in the gall wood vary from 1 to 3 and even 4 cells. The much greater volume of the cells in abnormal wood increases the significance of the discrepancies indicated above.

7. Increase in size and number of the fusiform rays comparable to that noted for linear rays.

8. The radial rows of xylem and phloem lose something of their orderly appearance as seen in cross sections (Pl. II, 1), a phenomenon noted in a lesser degree in normal wood making fast growth.

9. Pitting of the tracheids is found on the tangential as well as the radial walls (Pl. III, 5) to which it is considered limited in normal wood of *Pinus sylvestris* (10).

10. Cell walls of abnormal tracheids vary in width but often are twice that of the normal. Walls of the elements produced at the end of the growing season do not differ appreciably in thickness from those of the spring wood and the diameter of the cells is much the same throughout the annual ring, so that these rings are often difficult to distinguish in gall wood.

Discussion of Anatomical Studies

Meinecke (8) and Klebahn (7) have shown that repeating pine rusts may reinfect their pine hosts through the young epidermis of the current season's shoots. In the present study, as in the work of Hutchinson (6), the actual penetration of the epidermis by aecial germ tubes was observed.

While no detailed histological studies have previously been made of galls formed by the Woodgate *Peridermium,* the galls of *Cronartium quercuum* (Berk.) Miyabe (*C. cerebrum*) have received considerable attention. Weir (12) considered the annual growth of the galls to be stimulated by the presence of the perennial mycelium of the fungus in the living sapwood. Dodge and Adams (4) found the mycelium of *C. quercuum* (*C. cerebrum*) in galls on *Pinus rigida* Mill. to be intercellular and uninucleate, abundant in the cortex and apparently following the rays in the phloem and xylem. Haustoria were constricted where they penetrated the cell wall, were found to be abundant in the rays, common in the phloem, and comparatively scarce in the primary cortex.

Stewart (11) found that many of the tracheids in the gall wood of *C. quercuum* (*C. cerebrum*) had blunt ends and were differentiated from the wood parenchyma cells only in their pitting, which, at times, was irregularly 'Araucarian,' with the bars of Sanio seemingly lacking. The pits varied greatly in size and bordering, even in the same tracheid. Nonfusiform rays were often 2 to 3 cells wide, abnormally abundant, and composed of unusually large cells. Three times the usual number of resin canals appeared in cross

section, often so close that only the ray cells separated them. Above and below the gall, the normal number was present.

The present study has indicated the similarity of the galls produced by the Woodgate *Peridermium* to those of *C. quercuum* (*C. cerebrum*) as described by Stewart. He, however, interpreted the discolored nonconducting core as resinous in nature, while, in the case of the Woodgate *Peridermium,* the discoloration is probably due to tannin derivatives. Resin was found limited to the canals and did not impregnate the wood in any general fashion. It is well to bear in mind, however, that the term "tannins" as used in this paper includes the broad range of substances that give the "tannin" reaction with ferric chloride. Unlike the galls studied by Stewart, those of the *Peridermium* at Woodgate can, and often do, influence the structure of the host beyond them where no mycelium is present. Beyond the gall, the stem is often of larger diameter than below it, probably because of the fact that the abundance of phloem parenchyma in the gall has cut off much of the normal downward passage of elaborated material, which expresses itself in this diameter increase. With this diameter increase goes an increase in the number of resin canals. At times a tendency toward brooming may arise beyond or on the distal portion of the gall.

EFFECT OF GALL DEVELOPMENT UPON CONDUCTION THROUGH THE XYLEM

On August 18, 1933, conduction experiments were made with eosin to determine in some degree the effect of the gall upon the conduction of water

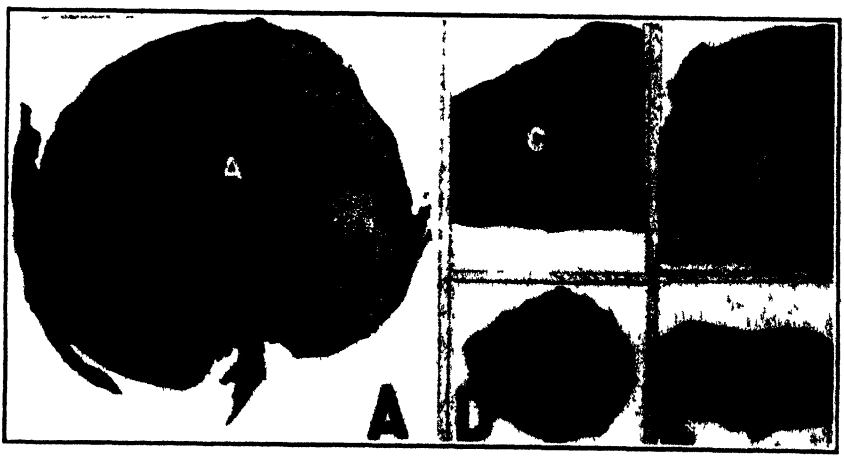

FIG. 4. A. Transverse section through an unstained gall showing dark color of the core (A) and wide area of conducting tissue (B) between it and the cambium at (C). B and C. Longitudinal sections through galls through which eosin has been conducted, staining the conducting tissue as at A and B. Note the contrast between the conducting eosin-stained tissue and the non-conducting naturally discolored core (C) and the nearly complete absence of conducting tissue between the core and the cambium of gall C. The branches beyond A and B appeared normally vigorous while that distal to C was dwarfed and dying. D. Young gall in which whole of gall wood still functioned in conduction and showed the eosin stain. E. Young gall in which the core failed to show the stain as strongly as the outer portion and whose core is inferred to have become at least partially non-conducting, though as yet no natural discoloration had appeared in it.

and dissolved substances to the branch beyond. One-, 2-, and 3-year-old portions of stems, with and without galls, were cut off below the surface of an eosin solution. The galls and noninfected areas of the galled twigs and normal twigs were examined when the eosin reached the tips of the current season's wood, and the stained and unstained areas were noted. In this way the presumably water-conducting wood, stained pink, was differentiated from the nonconducting which remained unstained.

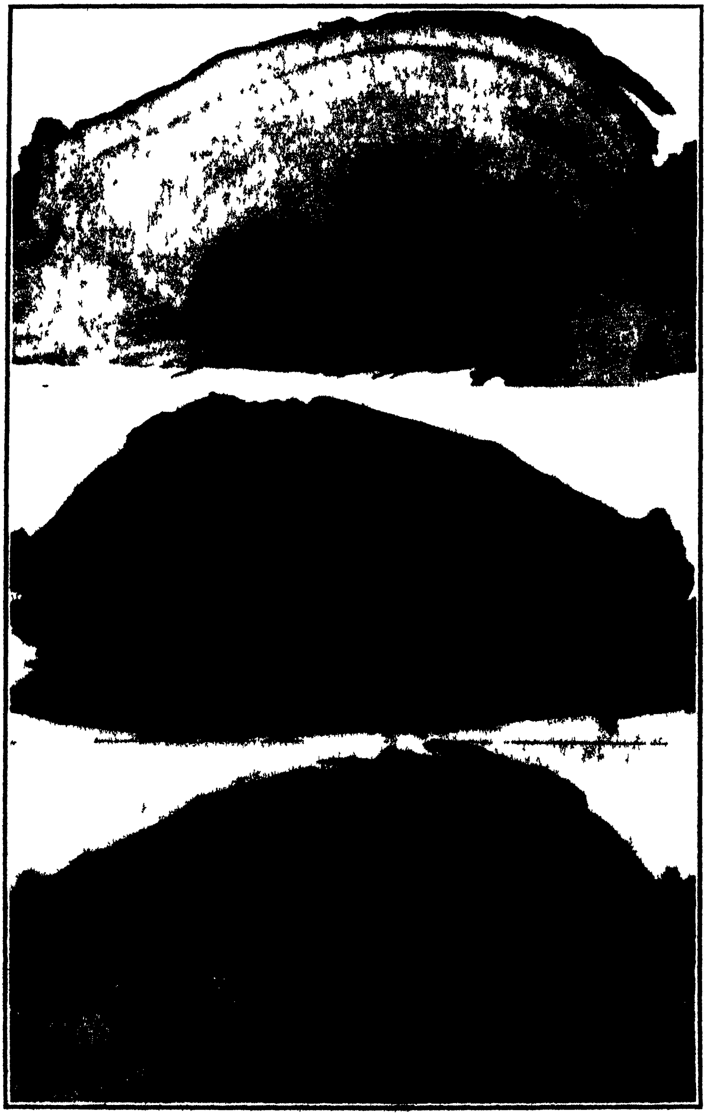

FIG. 5. A. Longitudinal section through untreated gall showing discolored core at center. B. Similar section of gall tested for tannins showing the positive tannin reaction of the core. C. Similar section of gall tested for starches showing the positive starch reaction of the woody tissues external to the core and the absence of starch from the core itself.

While all the gall wood of the 1-year-old swellings was active in conduction (Fig. 4, D), an undyed, or lightly stained, central core often appeared in 2-year-old galls (Fig. 4, E). In some of the 2-year-old and all of the 3-year-old galls, the cells of this nonconducting core were filled with brownish substances (Fig. 4, A). These cells and nondiscolored cells close to them contained tannin-like compounds instead of the starches and oils so abundant in the parenchymatous cells of the major part of the nondiscolored gall wood (Fig. 5, A, B, and C). In three-year-old normal stems, however, the whole woody cylinder was stained, and all the tracheids seemed to have been active in conduction.

The nonconducting discolored core of mature galls may be relatively small (Fig. 4, A and B) or may extend outward from the center of the gall and include all the cells of the gall wood except those most recently derived from the cambium (Fig. 4, C). If the first condition exists, the galls may cause little or no abnormality beyond the areas of local swelling. In the second condition, however, the branch beyond the gall may often be dwarfed or dying.

ASH ANALYSES[5]

In the hope that analyses of the ash of galls and rust-free portions of susceptible trees would perhaps indicate some differentiation between the physiological phenomena of gall formation and normal branch development, analyses were made on December 1, 1933, of fresh material from susceptible trees that was divided into two lots. The first contained galls from 2 to 4 years old, and the second consisted of the rust-free portions of the same branches from which the galls were taken. Bark and wood were taken together in both samples. These lots were ashed and analyzed by the method of H. S. Washington.[6]

TABLE 1.—*Results of ash analyses of 2 and 4-year-old galls and rust-free portions of stems of Pinus sylvestris*[a]

Constituent	Rust-free portions		Galls	
	2 Yrs.	4 Yrs.	2 Yrs.	4 Yrs.
CaO	17.76%	26.39%	18.57%	22.88%
MgO	5.38	7.22	7.25	6.50
K_2O	35.03	28.39	30.62	25.90
Na_2O	4.46	2.64	1.58	4.29
N	0.51	0.31	0.70	0.95
SO_3	5.33	4.10	5.15	7.46
P_2O_5	13.09	10.83	19.68	18.59
SiO_2	15.54	11.28	7.99	6.09
Fe_2O_3	2.92	3.37	2.68	2.72
Al_2O_3	0.53	6.10	5.75	5.34
MnO	0.41	0.03	0.18	0.12
Cl	0.2	trace	0.10	trace
% Ash	1.31	0.65	1.38	0.88

[a] Results reported on the basis of a carbon and CO_2 free ash.

[5] The author wishes gratefully to acknowledge the research grant from the University of Pennsylvania Chapter of Sigma Xi, which enabled him to have the analyses made.

[6] Analyses were made by H. J. Hallowell, consulting chemist, Philadelphia, Pennsylvania.

Table 1 shows some divergences between the ash of the galls and the rust-free wood. The greater amounts of nitrogen, sulphur and phosphorus in the galls can perhaps be correlated with the large quantities of food substances stored in them. The lesser amounts of silicon present in the galls appear to add a chemical reason to the structural basis of the mechanical weakness shown by gall wood. Most of the results, however, show differences whose explanation is at present difficult. Perhaps when the complexities of the physiology of the host-parasite relationship are better understood these figures may appear more significant but here the methods of ash analysis appear to shed little light on the physiological aspects of the problem.

RÉSUMÉ OF THE LIFE CYCLE AND MORPHOLOGY OF THE WOODGATE PERIDERMIUM

The Woodgate *Peridermium* is autoecious and has but 2 known spore forms, pycnia and aeciospores. The pycnia were first found during the course of this study. They were discovered in material collected on May 17, which had been selected to show developmental stages of the gall and, in choosing and fixing the material, the pycnia had been passed over unnoted. Later, other inconspicuous internal pycnia were found in sections of material from several young galls collected in the same season some of which had shown external traces of exudate at the time of collection. The aeciospores reinfect the host directly. They are roundly and irregularly rhomboid with verrucose walls. No germ pores have been observed in the walls of the aeciospores. (Fig. 3, D). They may germinate on water or upon the host in from 12 to 24 hours. The germ tube protrudes often but not always from an apex of the spore and broadens to form a septate branching hypha, which, within the inoculation chamber, may apparently penetrate the host at once or grow luxuriantly over the epidermis for a considerable distance before entering.

In most cases the mycelium vegetates within the host for 2 seasons and in May of its third season may produce pycnia from small hyphal wefts beneath the periderm of the central sunken portion of the gall often near its edge (Pl. III, 3). In late May and early June of the fourth season, approximately 36 months after infection and in successive seasons thereafter, aecia are produced. They arise from larger and denser wefts arising deeper in the tissue than the pycnial wefts and presumably radially interior to them, though the exfoliation of the pycnial weft precedes aecia formation. As the aecial weft develops, the host cells within and adjacent to it are separated widely from each other, but often do not appear to die for sometime, in consequence. The peridial cells of the young aecium push up the periderm and the upper portion of the hyphal weft, which may then disappear entirely or remain partially adherent to the edges of the mature aecium. The peridium varies from 1 to 4 cells in thickness (Fig. 6, A and Pl. III, 1) and stalactiform structures of the nature of the peridium are sometimes found uniting the dome of the peridium with the base of the aecium (Pl. III, 2).

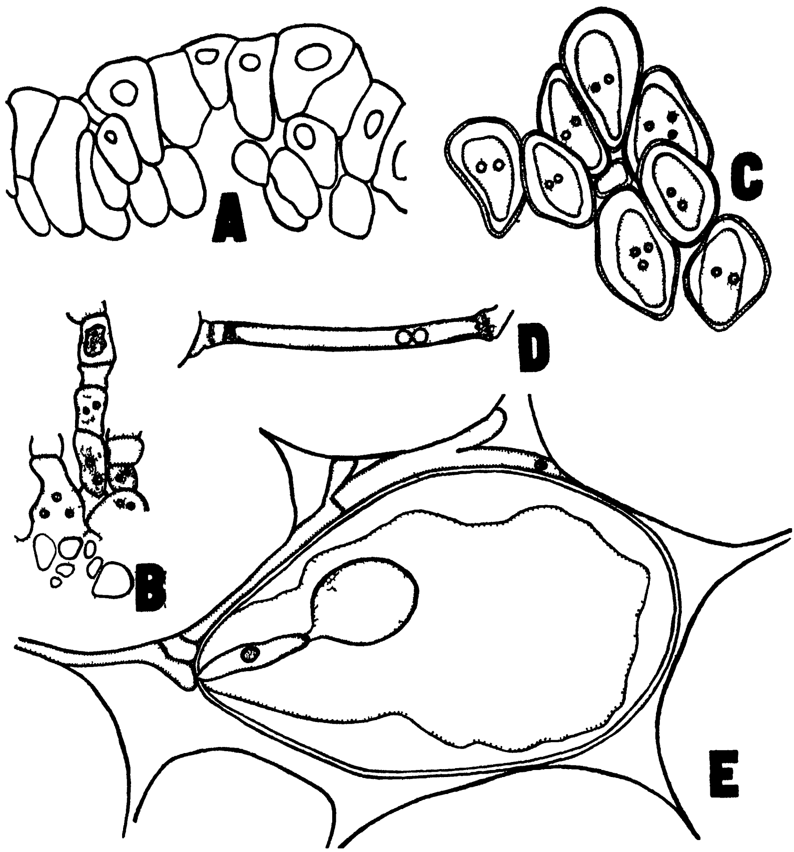

FIG. 6. A A portion of the dome of the peridium with cells drawn in outline. B. Portion of the bases of aecial spore chains showing binucleate and trinucleate condition. C. Young aeciospores, some binucleate and some trinucleate. D. An apparent binucleate condition in a cell of a vegetative hypha. E. Hyphae and haustorium in the cortex. Note the effects of shrinkage upon the nucleus at its point of contact with the haustorium.

The usual number of nuclei in the vegetative cells and of those making up sterile portions of the wefts is 1, and, while 2 have been exceptionally found in vegetative cells (Fig. 6, D), it seems likely that they do not indicate the initiation of a distinct stage in the life cycle.

A 2-, 3- and, exceptionally, 4-nucleate condition arises in the basal cells of the enlarged hyphae that cut off the young aecial spore chains (Fig. 6, B), and the young aeciospores are in turn usually binucleate but often possess 3 (Fig. 6, C) and at times 4 nuclei. The nuclei in maturing spores apparently become grouped, but no actual fusion has been observed. The young germ tubes, however, often appear to have 2 or more nuclei per cell, but only 1 has been observed in each cell at the time of host penetration. Until further cytological studies can be made upon this organism, the interpretation of these nuclear phenomena would appear hypothetical.

Dodge and Adams (4) found that of a collection of *Cronartium quercuum* (*C. cerebrum*) galls on *Pinus rigida* from New Jersey, some produced only

aecia and others only pycnia per season, and concluded that aecia and pycnia probably were produced on these galls in alternate years. In the case of galls on *P. virginiana* Mill., however, both aecia and pycnia developed on different parts of the same gall in the same year. While galls of repeating western rusts may produce aecia annually, Gill (5) reports that their pycnia are exceedingly rare.

GENERAL DISCUSSION

The Woodgate *Peridermium* is known to be definitely autoecious at Woodgate, New York.[7] The fact that its aecia produce germ tubes that reinfect the host without the production of sporidia precludes the possibility that we have here to do with the microtelia of a typical microcyclic rust. This *Peridermium,* then, in the character of its life cycle, seems only comparable to the two western repeating forms studied by Meinecke (8) and referred to by him as *Cronartium harknessii* Meinecke and *Peridermium cerebroides* Meinecke, and now grouped together by Arthur (2) as forms of *C. coleosporioides* (D. and H.) Arth. and to *P. pini* (Willd.) Kleb., a European repeating pine rust that does not form galls studied by Klebahn (7). The Woodgate *Peridermium* seems, however, to produce pycnia with greater frequency than either of the western forms, and its peridium, 1 to 4 cells in thickness, contrasts markedly with that of the repeating aecial form of *C. coleosporioides* (*C. harknessii*) whose peridium is 5 to 7 cells in thickness (1).

Intracellular penetration was found more frequently than penetration between epidermal cells. The early intracellular development of the mycelium in the epidermis and its formation of atypical, branching, septate haustoria in the subepidermal cells suggest the findings of Pady (9), who has shown the early tendency of infections toward intracellular development to be even more pronounced in the short-cycle orange rust of *Rubus.*

The coincidence of the paths of early, unresisted cortical invasion with the regions more richly supplied with starches and oils seems to signify that there may be a relationship between the amounts of food available to the rust and the supply of demonstrable starches and oils. It is interesting to note that the tannin-like compounds, present in normal quantities in the vacuoles of many cells in these regions, do not interfere with the migration of the mycelium along these paths.

Bearing in mind the possible relationship of potassium to foods available to the rust, Hutchinson (6) considered that resistance varied with the host and found that trees whose ash contained little potassium were more susceptible than those with larger amounts of it. He considered that infections that fail to produce galls in susceptible trees did so primarily through competition and lack of nutriment. In this paper the view is taken that, at Woodgate, susceptibility varies not only with the host but also with the parasite, and that there is a question of compatibility involved between the host and each mycelium whose germ tube penetrates it. It may well be borne in mind, too, that the entrance of secondary organisms, some of which have

[7] H. H. York. The Woodgate *Peridermium.* [Manuscript.]

quite frequently been noted in histological preparations of young galls, might influence the reaction of otherwise compatible hosts.

It is true, as Hutchinson has pointed out, that different parts of the same tree may produce galls more readily than others following infection. The host tissue of a single shoot is a variable factor, since portions of the young stem, such as areas at the needle bases, may be better supplied with starches, oils, etc., than others. Yet it seems unlikely that a commandeering of the food supply by the first mycelium to penetrate could explain the wide variation of host reaction to the different invading mycelia in inoculated twigs where the infections may be considered to have taken place at about the same time. In these cases, too, as in others studied, there seems to have been no relation between the compatibility of the mycelium with its host and the region of the shoot attacked, though definite regions of abundance and scarcity of demonstrable stored foods do exist in the shoot as indicated. Also, areas invaded by incompatible mycelia and those adjacent to them, rather than being free of demonstrable foods, are abnormally full of starch and oil in the early stages of invasion.

Compatibility in susceptible trees, then, seems to vary not only with the host but with some property of the infecting mycelium. That this variable property is a physiological one seems clear. It appears likewise unlikely that a variation in vigor of the invading mycelium is the sole cause of the variation in the host reaction. Rather, it seems likely that some mycelia are genetically better adapted than others to achieve and maintain the metabolic balance with the host so necessary to obligate parasites. Perhaps the varying number of nuclei in the aeciospores may indicate such an instability in the genetic constitution of the *Peridermium* at Woodgate. The question is here raised whether we do not, then, have in this case of a forest tree rust something comparable to the physiologic races recognized among cereal rusts.

Even in the cases of most complete compatibility, the cells of all the invaded areas except the cambium eventually die and, in contrast to phenomena sometimes exhibited in the cereal rusts, die sooner than cells of comparable but uninvaded tissues. This is true of the invaded cortical tissues as well as of the phloem and xylem. The length of time intervening between invasion and death of the cortical tissues usually measures the compatibility of the reacting organisms and consequent susceptibility of the host, since an infection that reaches the cambium seldom fails to cause gall formation. The more compatible mycelia at first cause but little abnormality in the structure or contents of invaded parenchymatous host tissues, but gradually these become filled with starches and oils and, just previous to death of the cells, unusually large amounts of tannins appear in them in the place of the oils and starches. Where compatibility is less, this series of events follows in swifter succession. Coincident with, or shortly after the appearance of an abnormal abundance of tannins in cortical cells, less compatible mycelia meet the resistance of hypertrophy or of hyperplasia or both and the degree of structural abnormality of the tissues is proportional to the physiological

abnormality expressed in terms of host cell contents. Coincident with the death of the cells in the invaded area, a row of meristematic cells arises to form a cicatrix, which proves to be a barrier to invasion only when death of the cells occurs so quickly after invasion that the mycelium has no time to grow beyond the zone of cicatrization.

Only cambial initials in close proximity to the hyphae are influenced by the presence of the organism to produce abnormal phloem and xylem elements. Parenchymatous cells are produced in much greater proportions than normal and these are soon filled with abnormally large amounts of starches, oils, and, in the case of the phloem parenchyma, tannins. Thus, the parasite appears to cause its host not only to provide it with extra food but also with extra food-containing cells. These abnormalities are most pronounced in tissues derived from newly invaded cambium, and there is some suggestion of a return toward the normal in tissues arising from initials in cambium areas that have been invaded for a year or more. After periods of time, which vary in different mature galls from less than 1 to as much as 3 years after their formation, invaded tracheary xylem elements cease to conduct, the starches and oils are replaced by tannins, some of which seem to be located at the periphery of the cells, the cells die, and the mycelium in the affected area dies with them. When death occurs too soon after cell formation, *i.e.*, too close to the cambium, so that the number of conducting cells is much below normal, the branch beyond the gall often shows dwarfing and may die probably, at least in part, because of water shortage. The distance between the cambium and this nonconducting core may be a further measure of compatibility.

SUMMARY

The life cycle of the Woodgate *Peridermium* has been followed histologically from penetration of the epidermis of the current season's shoot of *Pinus sylvestris* by aecial germ tubes through gall formation to production of pycnia 2 years after penetration and repeated formation of aecia in ensuing seasons.

The wide variety of early response of single susceptible twigs to invasion suggests the existence of more than one physiologic race of the *Peridermium* at Woodgate.

Mycelia causing little early abnormality in the tissues invaded are considered as compatible with the host. These penetrate the cortex more swiftly along characteristic diverging paths found to possess demonstrable nutrients in greater abundance than areas traversed more slowly.

In the case of mycelia extremely incompatible with the hosts, starches, fatty substances, and, finally, tannin-like compounds are rapidly concentrated in cells near the hyphae. Following the deposition of abnormally large quantities of the last, these cells die and the fungus dies with them.

The more compatible mycelia grow out in advance of this killing and succeed in forming galls, but cell hypertrophy, hyperplasia, and barrier formation often hinder their passage through the cortex.

Compatible mycelia invade the matured secondary tissues by the end of

the first season, while others may be delayed. Phloem and xylem are invaded mostly along medullary rays. Stelar tissues, matured before invasion, show comparatively slight structural change, but their parenchymatous cells become abnormally full of starches, fatty substances, and tannins.

The presence of the fungus in the cambium causes the production of abnormal secondary tissues containing a high proportion of parenchymatous cells that become in turn filled with starches, fatty substances, and, finally, tannins. Increase in cell size characterizes both phloem and xylem, while only the latter shows a marked increase in cell number. Irregularities in size, shape, and pitting of the tracheids also are produced.

Portions of the cambium penetrated for the first time produce the most abnormal secondary tissues, so that areas of gall wood are outlined by tissues showing the greatest abnormalities.

Abnormal parenchyma tissues in the xylem at first contain large amounts of starch and fatty substances, which are replaced by tannin-like compounds usually during the third season in the oldest gall wood, which then ceases to conduct. In cases where the nonconducting core occupies nearly all of the xylem, the branch beyond the gall often dies.

Meanwhile, the death of the inner cortex and of the abnormal phloem cells leaves portions of the cortex external to them isolated from radial conduction. These portions die, collapse, and later are exfoliated except at the edges, where, by contrast, they appear as a raised collar.

Early in May 2-year-old galls often bear pycnia hidden directly beneath the periderm below the sunken central area. This is the first report of pycnia for the Woodgate *Peridermium*.

Three-year-old galls often produce confluent aecia from extensive dense mycelial wefts in the sunken area arising slightly below those of the then exfoliated pycnia Each year the tissues containing the aecial weft are exfoliated by a periderm arising internal to them and the next year aecia are formed below the new periderm.

Ash analyses of 2- and 4-year-old galls and rust-free portions of susceptible trees showed the galls to be significantly lower in silicon and higher in nitrogen, phosphorus, and sulphur. Other discrepancies were slight or difficult to interpret.

Preliminary nuclear studies revealed the perennial vegetative mycelium to be predominantly uninucleate. Two, 3 and occasionally 4 nuclei appear in the cells at the base of the aecial chain, the young aeciospores, and many portions of the germ tubes. The latter, however, are markedly uninucleate when they penetrate the host.

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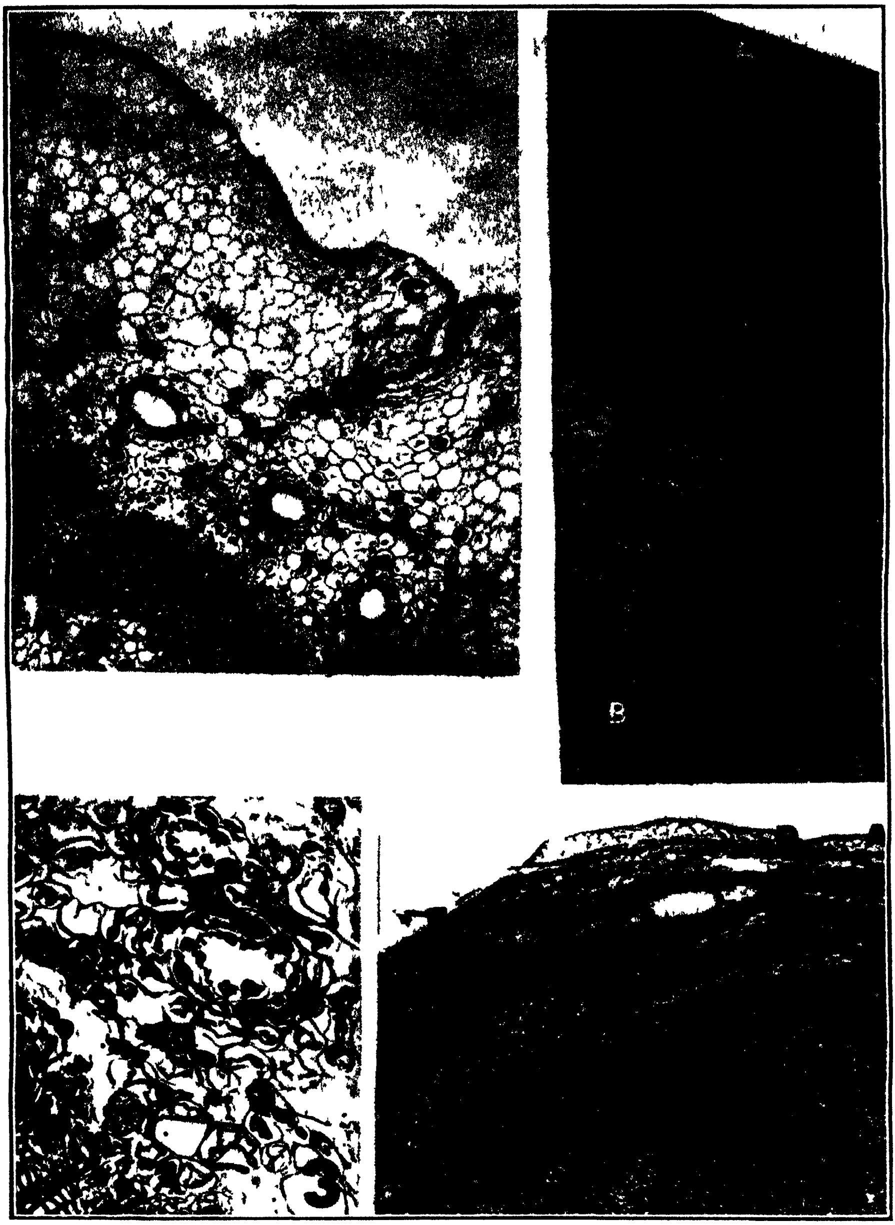

PLATE I

1. Transverse section of a normal twig of *P. sylvestris* as it appears in early June. Note especially the thickness of the walls of epidermal and subepidermal cells at A, the base of the needle fascicle supplied by the bundle trace at B as contrasted with the thinner walls elsewhere as at C. 2–3. Transverse sections showing unresisted invasions. 2. shows a very limited necrosis at point of penetration (A), scarcity of hyphae in the outer cortical region as compared with the inner, especially in the vicinity of the resin canal and the closing of the canal by the hypertrophy of the epithelial cells. The mycelium is shown entering the stelar tissues through a bundle trace at B. 3 shows a resin canal still open in spite of the abundance of hyphae near-by. The mycelium enters the phloem of the main stele at A. 4. Early stage in the formation of the "collar". Necrosis has spread from near the center of infection (A) largely in the inner cortex to B and this, together with pronounced abnormalities arising in the inner cortex and phloem from B to C, has partially isolated the outer cortex external to it. Following its isolation, it has become severely necrotic from A to D and appears to be dying also from D to E where a barrier from D to C is forming to separate the dying portion from the more normal cortex at the left in the photograph.

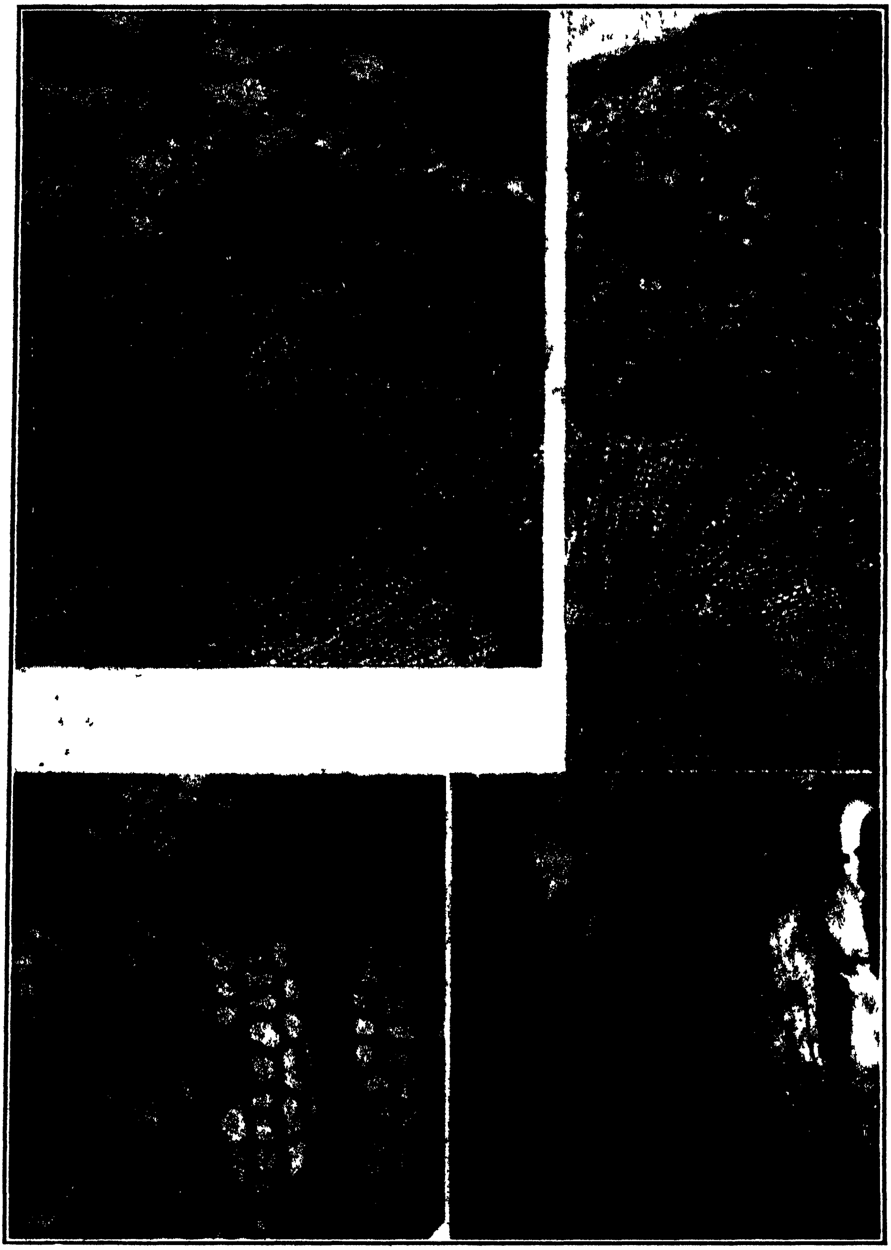

PLATE II

1. Transverse section showing the edge of an infection with invaded and uninvaded secondary tissues side by side. Note that in the pathic phloem the parenchyma cells with their deeply staining contents (A) have almost completely displaced the sieve tubes (B), which comprise most of the phloem in uninvaded tissues. 2. A cicatrizing zone formed from A to B to C to D has proved ineffectual in resisting the invasion as has the similar zone from A to E to F, which formed later. Note that the mycelium is already present in the phloem G and xylem H. Radial resin duct in uninvaded tissue uniting the resin cavity A in the phloem with the vertical xylem resin duct. 4. Radial longitudinal section showing edge of infection where the mycelium is advancing vertically and radially into uninvaded phloem (A) and crossing the cambium to enter the very abnormal xylem. Note that abnormalities in the secondary tissues are produced at some distance in advance of the mycelium. 5. Transverse section showing mycelium crossing the cambium and distributed mostly radially in pathic phloem and xylem though not always confined to the rays.

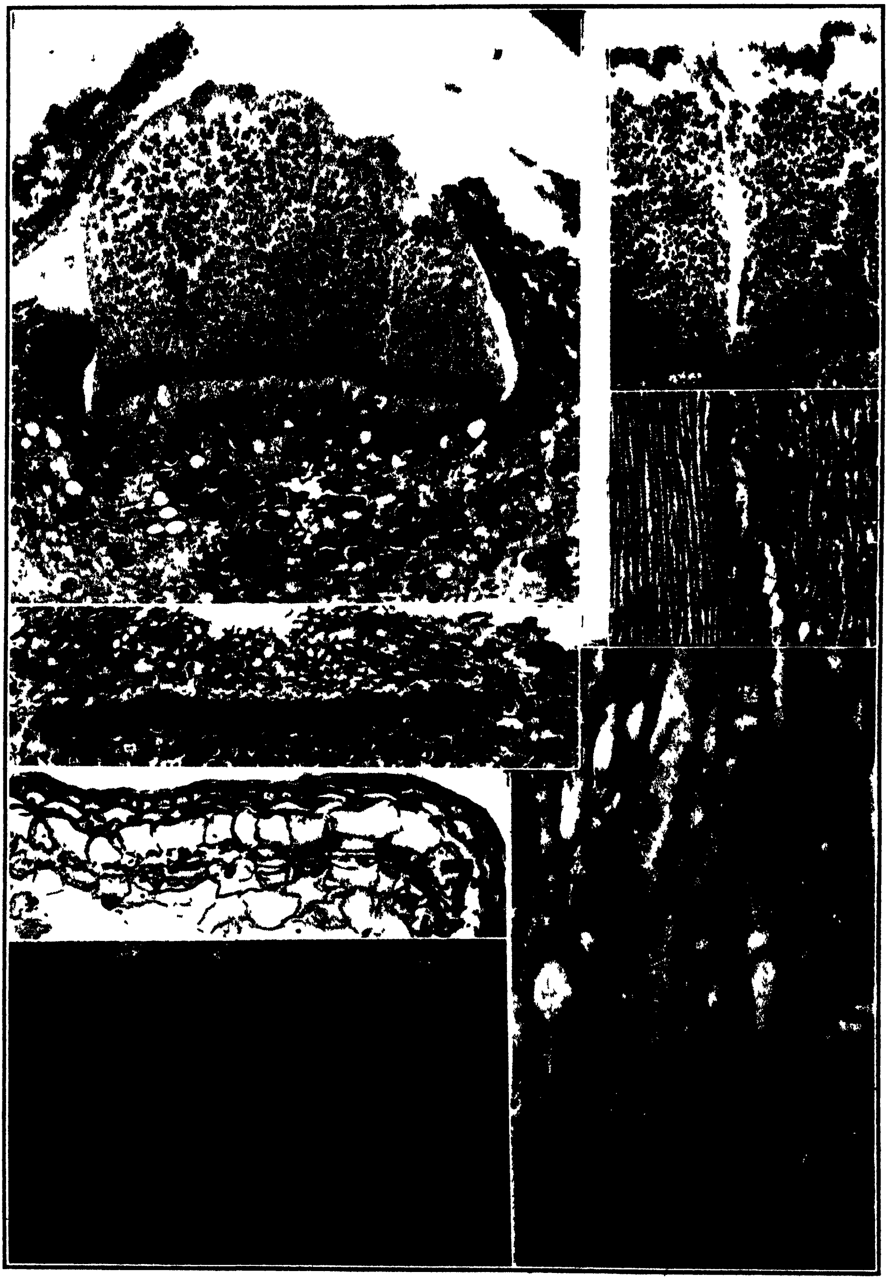

PLATE III

1. Section through a small aecium showing external periderm, the peridium of the aecium, the darkly stained basal cells of the spore chains, and the widely separated host cells below the aecium. 2. Section through the filaments occasionally found showing their double character and suggesting that they are separations between otherwise confluent aecia. 3. Section through a pycnium showing the very limited pycnial weft and its position below the periderm. 4. An approximately tangential section through gall wood and uninvaded wood adjacent showing the irregularities and large size of the cells of the gall wood and their disproportionately large amount of parenchymatous tissue. 5. A higher magnification of a portion of the gall wood shown in 4, showing particularly the tangential pitting of one of the walls of one of the tracheids at A. 6. Radial longitudinal section showing the mycelium oriented radially in and near the cambium. 7. Epidermis and periderm of normal *P. sylvestris* as they appear in early August.

FAILURE OF DASYSCYPHA WILLKOMMII AND RELATED LARGE-SPORE SPECIES TO PARASITIZE DOUGLAS FIR

GLENN GARDNER HAHN AND THEODORE T. AYERS

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INTRODUCTION

Soon after the discovery of the European larch canker, in 1927, in Massachusetts (8), Douglas firs, *Pseudotsuga taxifolia* (LaM.) Britt., growing adjacent to the diseased European larches, *Larix europaea* D. C., were reported as being attacked by this introduced European disease. The basis of this report was the finding on cankered Douglas fir, of a large-spore *Dasyscypha*, which at the time appeared to agree with available descriptions of the larch canker fungus. This parasite had been reported in European literature as attacking Douglas fir (5, p. 900).

There was also present on the Douglas fir canker, a related *Dasyscypha*, the identity of which, and its relationship to the European larch canker organism, was uncertain at the time. In a separate investigation, this unidentified *Dasyscypha* was found to be a native species, *D. ellisiana* (Rehm) Sacc., commonly present as a saprophyte on species of pine in the east. Artificial inoculations, on which a preliminary report has been published (5), have demonstrated that this native *Dasyscypha* in some cases is capable of acting as a weak parasite and of causing canker of Douglas fir. The investigation has shown, however, that *D. ellisiana* is not entirely responsible for the diseased condition of Douglas fir in New England.

To determine whether Douglas fir would be attacked by the true larch canker organism, *Dasyscypha willkommii* (Hartig) Rehm, and related large-spore species (4), namely, *D. calycina* Fckl., introduced from Europe, and the native species, *D. oblongospora* Hahn and Ayers and *D. occidentalis* Hahn and Ayers, inoculations were made with these organisms on this conifer at different times from 1931 to 1934. The inoculations were supplemented by a field search for the larch-canker fungus on Douglas fir, not only in the area where it was discovered on imported larch (8), but also in other localities in New England. Previous to this study in the United States, unfruitful search for the larch-canker fungus had been made in Great Britain on Douglas fir growing in pure plantations and in mixed plantings with European larch. In Britain, larch canker has been present many years and is found attacking larches growing intermixed with Douglas fir.

The results obtained from these studies have a direct bearing on the planting of Douglas fir in the United States and in European countries where the larch canker is established and widespread. Moreover, general statements recently published by us (5, p. 901) concerning the failure of the European larch-canker organism to parasitize Douglas fir under American conditions, are substantiated by detailed experimental and observational evidence described in this paper.

FIELD SEARCH FOR DASYSCYPHA WILLKOMMII ON DOUGLAS FIR

In Europe

A brief, preliminary field study of planted Douglas fir in Scotland with regard to possible infection by the European-larch-canker organism was made by the senior author in 1928 and 1929. These observations were confined to plantations at Glentress above the town of Peebles, in the Valley of the River Tweed, Peeblesshire. At an elevation of 400–500 feet there were small plantations of Douglas fir, both green and blue forms, varying in age up to approximately 20 years.

One of these consisted of a mixture of the green form with Norway spruce and European larch in which the European-larch canker was causing severe injury to the latter species. The moist conditions prevalent in this stand, which was closely crowded, were particularly favorable for the propagation of the larch-canker fungus and related Dasyscyphae, which were fruiting abundantly on larch. Such a situation proved to be an excellent one in which to search for *Dasyscypha willkommii* on rapidly growing, dominant, and stunted, suppressed green Douglas fir, among which the D. B. H. varied from 3–7 inches.

After careful search among several hundred trees, in no instance was a single Dasyscypha canker on the Douglas fir found resembling those so commonly present among the diseased European larch. However, infrequently, a very few, small, inconspicuous fruit cups of *Dasyscypha,* resembling macroscopically the European-larch-canker organism, were found growing on weakened, suppressed, lower branches which were either dead or in the process of dying. Later, a microscopic and cultural study of this *Dasyscypha* species, found to occur saprophytically on larch as well as on Douglas fir, showed it to be distinct from that parasite. As in the case of European-larch-canker organism, this *Dasyscypha* produced large elliptical spores, but these were not pointed as in the case of some of the ascospores of *D. willkommii.* This Douglas fir Dasyscypha, which might be confused by some with the true larch- canker fungus because of its large elliptical spores, has been referred (4) to the species *D. calycina* (not *Peziza calycina* Schum.).

Furthermore, an examination of a blue Douglas fir plantation, in which some of the green form was growing not far distant from the mixed stand just described, did not reveal Dasyscypha cankers nor fruiting of the fungus. The vigor of trees of the blue form had been reduced by *Rhabdocline pseudotsugae* Syd., which was prevalent and causing a serious needle cast.

Another young plantation[1] of trees of the green form, planted in 1923

[1] Baxter, D. V. Observations on forest pathology as a part of forestry in Europe. Michigan Univ. School of Forestry and Conserv. Bull. 2. 1933. There is a photograph of this plantation in Pl. IV, fig. 1. Baxter does not report the occurrence of *Dasyscypha* on Douglas fir in this planting. In Pl. VIII he shows badly deformed and cankered trunks of European larch caused by *Dasyscypha willkommii,* growing in an open stand near Peebles. This plantation adjoined the mixed one of larch, spruce, and Douglas fir referred to in the foregoing. Note the stunted green Douglas fir in the foreground.

(about 8 years old) in the same general area, had suffered seriously from frost injury that had killed back the terminals, giving the trees a stunted appearance. This plantation was, likewise, free of larch-canker organism. In this instance 2 species of *Phomopsis* following frost killing, *Phomopsis occulta* Trav. and *P. conorum* (Sacc.) Died., were identified by the senior writer as being commonly present.

It is of interest here to note that Boyce (1), who made a survey of the diseases of Douglas fir in Great Britain in 1925, also visited and made studies in the Peeblesshire area just described. He did not report the European-larch-canker organism as either a parasite or a saprophyte on *Pseudotsuga taxifolia.*

The senior writer's experience in searching for the European-larch-canker fungus on Douglas fir in Scotland corroborates that of Plassmann (7) in Germany, who, likewise, sought for the larch parasite on that species. In that country, where larch canker in the past has been a serious disease and where Douglas fir has been planted, Plassmann was unable to find *Dasyscypha willkommii* acting either as a parasite or a saprophyte on that conifer, although the tree species was frequently grown in close association with diseased larch, making the spread of spores of *D. willkommii* to the Douglas fir extremely easy. In his reply to the controversial question, "*Dasyscypha willkommii*—a new disease of the Douglas fir?" which was discussed by Trendelenburg (9) in a report to German foresters, Plassmann (7) stated that on the basis of his field experience in Germany the larch-canker organism was not to be feared as an enemy of the introduced forest tree.

In England, on the other hand, Day has collected what he regards as the larch-canker organism [which he called *Dasyscypha calycina* (Schum.) Fckl.] on Douglas fir as well as Scotch pine. Day submitted collections of these Dasyscyphae to Plassmann, who confirmed his determinations, but expressed the further opinion that "In beiden Fällen, und das muss hier ausdrücklich hervorgehoben werden, ist *Dasyscypha willkommii* aber reiner Saprophyt" (7, p. 367). In connection with the *Dasyscypha* collections made by Day on Douglas fir in England, which we have not seen and, therefore, have been unable to compare with our specimens collected on Douglas fir in Scotland and the United States, it is of interest here to note that he does not report the larch-canker organism among the secondary fungi associated with Douglas fir cankers commonly resulting from frost damage (2). This type of lesion would appear to the writers to be a very likely sort of place for the occurrence of a *Dasyscypha* form acting in no way as a parasite. On the other hand, Day (3) states in a later paper that the larch-canker fungus was present in the dead tissues of all the larch cankers of traumatic origin that he studied.

In the United States

An intensive search was begun in the autumn of 1929 for large-spore species of Dasyscyphae among the diseased trees of Douglas fir (6) growing adja-

cent to infected imported European larch at Hamilton, Massachusetts, where the 2 species had been growing together undisturbed for approximately 20 years. The great abundance of fruit bodies of *Dasyscypha willkommii*, which occurred on the larch in this particular area where diseased trees had not yet been removed, gave evidence that the European disease was on the point of becoming a serious outbreak.[2] Inasmuch as the site on which both European larch and Douglas fir were growing was highly favorable to the propagation of larch canker and the organism fruiting on the lesions, it was reasonable to expect that infection could have extended readily to the Douglas fir if this conifer had been susceptible.

A search in this plantation of Douglas fir from 1929 to 1933, which period included eradication of the adjacent diseased larches in 1932 and 1933, did not reveal fruit bodies of *Dasyscypha willkommii*. Moreover, the writers failed to find the larch-canker organism in other diseased Douglas fir plantations in New England some distance removed from the larch-canker infection area. Although there has been ample opportunity for Douglas fir to become infected during the time the larch canker was undisturbed, and before eradication had been undertaken, there is no evidence that the causal organism is able to attack species other than those of the genus *Larix* and its close relative, golden larch, *Pseudolarix amabilis* Rehder (5).

INOCULATION EXPERIMENTS

In order to learn whether the European larch-canker fungus, *Dasyscypha willkommi*, and 3 closely related large-spore Dasyscyphae, would infect Douglas fir, 143 artificial inoculation tests were performed with these fungi. The inoculations were made in the late summer and autumn of 1931, in the spring of 1932, in the winter of 1933 and in the spring of 1934, under controlled conditions in recently planted (1930) 8-year-old transplant stock of the Colorado or blue form of Douglas fir. Healthy, vigorous trees were utilized as well as some that were languishing and dying and some practically dead. The inocula used were derived from recently isolated cultures of the Dasyscyphae, procured from single asci or asco-spores growing on 3 per cent malt agar. The isolations of the larch parasite were obtained from collections taken in the infection area at Hamilton, Massachusetts. In a few inoculations, freshly collected fruiting bodies of the larch-canker organism itself were employed, together with bits of the diseased tissue of larch upon which they were growing.

The following technique was employed. The place to be inoculated was first cleansed with a cotton swab soaked in 95 per cent ethyl alcohol to remove débris clinging to the uninjured back. This was followed by a final alcohol wash for sterilization purposes. Incisions were made with a sterile scalpel by slitting the bark parallel to the long axis of the trunk or branch and gently raising the bark from the wood on both sides of the slit with the sharp

[2] Spaulding, P., C. B. Bidwell, J. S. Boyce, and A. W. Gottlieb. Report of committee on forest disease control. 3 pp. Presented at meeting New England section, Soc. Amer. Foresters, Boston, 1936. [Mimeographed.]

tip of the incision instrument. The entire incision was approximately 1 cm. long. The inoculum was then inserted into this with a sterilized needle, and the cut edges pressed down gently so that the wound was not only almost closed, but also the severed tissues were brought into direct contact with the inoculum. Finally the wound was wrapped tightly with a bit of cotton soaked in sterile distilled water, and the whole tied up in an outer wrapping of waxed paper that served both to conserve moisture and to aid the inoculating fungus in gaining a foothold for its initial growth on the injured tissues. Checks were made in a similar manner except in this instance only sterile agar was used.

A series of 18 Douglas fir trees were used for these tests, 6 of which were inoculated with 2 or more *Dasyscypha* species. In table 1 are given various details of the inoculations of current to 5-year-old wood of *Pseudotsuga taxifolia* with the 4 large-spore species of *Dasyscypha*. The places of inoculation were the trunk, main shoot, branch, or axil of a lateral branch and main axis. Checks were similarly placed but on wood that was 3 to 6 years old. Since none of the fungi were able to cause cankers, detailed reports of the individual inoculations do not seem justified.

In table 1, a total of 54 inoculations are reported for *Dasyscypha willkommii,* 35 of which were made with malt agar cultures and the remaining 19 with freshly collected apothecia of the larch-canker fungus growing on dis-

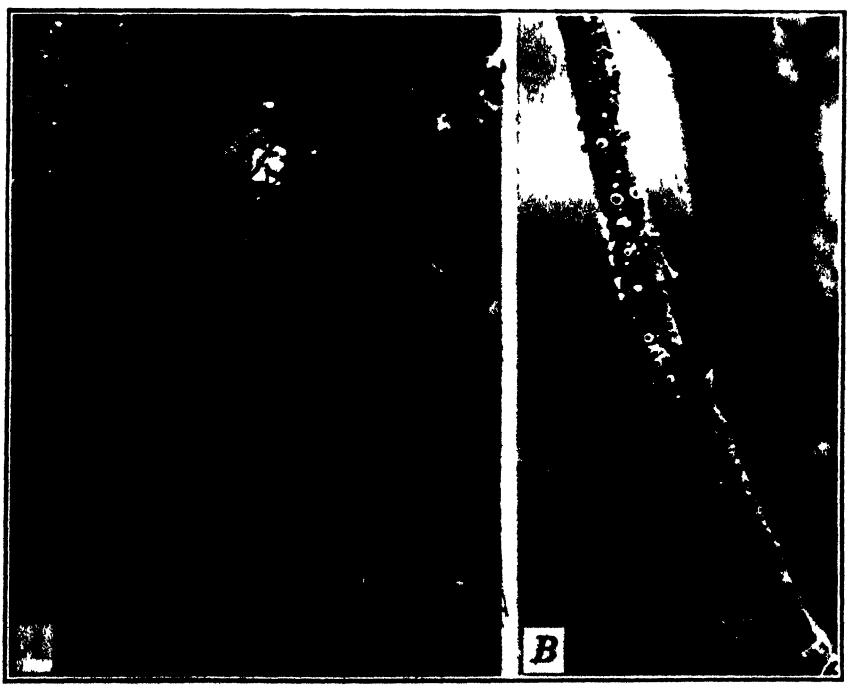

FIG. 1. Apothecia of *Dasyscypha calycina* Fckl. produced by a mono-ascus strain inoculated on dying Douglas fir. *A*. The arrow indicates the incision about which artificially produced ascocarps were produced. *B*. Enlargement of fruit bodies about the incision indicated in *A*. Approx. × 1.5.

eased larch tissue. Seven of these were made in a weakened tree and the remainder on 2 vigorous trees. In none of the inoculations was there any evidence that *D. willkommii* was parasitic or *saprophytic* on living or dead tissue of Douglas fir.

In the case of the *Dasyscypha calycina* inoculations, only agar cultures were used. As shown in table 1, 17 of these inoculations were made on healthy trees while 35 were placed on dying or dead trees. In only one instance, a mono-ascus strain of this species showed ability to colonize 6 incisions made August 13, 1931, on a single weakened Douglas fir, the needles of which were still green at the time of its inoculation, but the tree was not

TABLE 1.—*Inoculations of Douglas fir with Dasyscypha willkommii and related species*

Species of *Dasyscypha* and tree condition	Inoculation dates	Strains tested	Trees tested	Inoculated incisions	Cankers	Incisions colonized	Non-inoculated incisions	Cankers on non-inoculated incisions
D. willkommii:	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Vigorous	7	6	7	39	0	0	25	0
Weak	2	1	1	15	0	0	3	0
Total	9	7	8	54	0	0	28	0
D. calycina:								
Vigorous	4	4	4	17	0	0	13	0
Dying	4	5	5	23	0	6	6	0
Dead	1	1	2	12	0	0	6	0
Total	9	10	11[a]	52	0	6	25[b]	0
D. oblongospora:								
Vigorous	2	2	2	11	0	0	6	0
Dying	4	5	5	13	0	5	6	0
Total	6	7	7[a]	24	0	5	12[b]	0
D. occidentalis:								
Vigorous	2	2	2	7	0	0	6	0
Dying	3	3	4	6	0	0	3	0
Total	5	5	6[a]	13	0	0	9[b]	0

[a] A total of 18 Douglas fir trees were inoculated. Several of the trees were inoculated with the different fungus species: 3 trees in the *Dasyscypha calycina* tests were used for *D. willkommii*; 6 trees in the *D. oblongospora* tests were used for *D. willkommii* and *D. calycina;* 5 trees in the *D. occidentalis* tests were used for the other 3 species.

[b] A total of 56 check incisions were made. Eighteen of these checks served for more than one species of *Dasyscypha* inoculated on the same tree at the same time.

making new growth. The inoculum used in this particular case was obtained originally from a meager collection of *D. calycina* (F. P. 53056),[3] growing saprophytically on dead, noncankered branches of Douglas fir growing adjacent to diseased European larch at Hamilton, Massachusetts. *Dasyscypha calycina* gained a foothold in the dying tissue, and, after 2 years, colonies of the fungus had formed, producing small but normal fruit bodies on the underside of the branch, opposite the incision slit (Fig. 1, *B*) or in its immediate vicinity. These artificially produced apothecia did not extend more than 1.5 cm. above or below the incision, probably because of the fact that the

[3] Collection numbers denote specimens for study filed in the Division of Forest Pathology, B. P. I., New Haven, Conn.

Douglas fir tissues had become dried out (Fig. 1, *A*); and with this change in moisture relationship further spread of the fungus was inhibited.

The morphological characters of the artificially produced apothecia of *Dasyscypha calycina* were identical with those of fruit bodies of the fungus produced naturally on Douglas fir and larch in the larch-canker infection area. These characters have been described and figured in our paper (4) in which *D. willkommii* and *D. calycina* have been presented as separate species. In Britain, certain workers, as pointed out by Boyce (1, p. 12), regard these 2 species as members of a polymorphic species. They have recognized that the forms differ physiologically, but not morphologically.

A total of 37 inoculations were made with agar cultures of the native species, *Dasyscypha oblongospora* and *D. occidentalis*. In the case of the former, strains of *D. oblongospora* (53185), isolated from apothecia collected on Douglas fir in the Hamilton infection area, were able to colonize 5 out of 6 incisions made September 24, 1931, on a single dying tree of Douglas fir, which possessed green needles but had ceased putting on new growth. Three of the incisions were inoculated with a mono-ascus strain and three with a mono-ascospore strain. As in the case of *D. calycina* described above, normal apothecia formed within a period of two years from the time of inoculation with the mono-ascus strain. However, in the case of the other 3 inoculations with the mono-ascospore strain, 1 of which was negative, stromata of the fungus developed on the tissue about the incisions. In one instance, however, there appeared among the stromata a single fruit body that up to the time of examination, had not formed asci.

The morphological characters of the fertile, artificially produced apothecia of *Dasyscypha oblongospora,* as in the case of *D. calycina* discussed above, were likewise identical with those of fruit bodies occurring naturally on tamarack and other conifers. In both instances the experimentally produced ascospores were germinable, and the cultures obtained from them showed close agreement with those obtained from naturally produced ascospores of these 2 species. The fact that the morphological characters of artificially produced fruit bodies were similar to those of naturally produced ascocarps, indicated that the characters we had designated as descriptive of the species *calycina* and *oblongospora* (4) were constant for these species.

In our inoculation experiments, *Dasyscypha occidentalis,* which resembles rather closely *D. calycina,* failed to grow either parasitically or saprophytically on Douglas fir. The results of these tests corroborate our field observations, since this fungus has never been found on that conifer in either northeastern or western United States. In the Pacific Northwest, *D. occidentalis* is especially abundant on western larch, *Larix occidentalis* Nutt., growing in mixed stands with Douglas fir, but it never has been found on the latter species. In contrast, *D. oblongospora* (4), which is commonly saprophytic on larches and pine in eastern United States, has been collected a few times on Douglas fir in New England.

Although only a limited number of inoculations were made, we have been successful in demonstrating the colonization by *D. calycina* and *D. oblongo-*

spora of dying Douglas fir tissue. When one considers how rarely we found these species occurring naturally on similar tissue of that conifer, the results obtained are quite understandable. Furthermore, even though our experiments have not been very extensive to support the statement that *D. willkommii* will not colonize artificially weakened and dying Douglas fir tissue, field observations described in the preceding section have demonstrated that very probably this does not take place. Inasmuch as *D. willkommii* did not manifest saprophytism under natural conditions favoring such an occurrence, it does not seem very probable that we would have been successful in an artificial attempt.

SUMMARY

Artificial inoculations, supported by field observations, indicate that, in the United States, the European-larch-canker parasite, *Dasyscypha willkommii* (Hart.) Rehm, does not grow on the blue form of Douglas fir, either parasitically or saprophytically. Furthermore, the writers have found no evidence that this organism parasitizes Douglas fir in Europe.

Closely related saprophytes, the introduced *Dasyscypha calycina* Fckl. (not *Peziza calycina* Schum.) and the native *D. oblongospora* Hahn and Ayers, have been found fruiting rarely on the dead branches of Douglas fir in the area where *D. willkommii* was discovered on imported European larch and where Douglas fir was growing adjacent to the cankered larches. These 2 saprophytes were induced to grow upon the dying tissue of Douglas fir, but not upon living healthy tissue.

The morphological characters of artificially produced apothecia of *Dasyscypha calycina* and *D. oblongospora* were identical with those described for apothecia of these species produced naturally.

Another member of the large-spore group, the native *Dasyscypha occidentalis* Hahn and Ayers, has not been found growing either saprophytically or parasitically on Douglas fir, in nature or in the inoculations.

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TAXONOMIC RELATIONSHIPS OF PLANTS SUSCEPTIBLE TO INFECTION BY TOBACCO-MOSAIC VIRUS

FRANCIS O. HOLMES

(Accepted for publication Oct. 1, 1937)

For many years it was thought that tobacco-mosaic virus (tobacco virus 1) could infect only solanaceous plants. Later 2 susceptible species were recognized in other families. These were *Martynia louisiana* Mill. in the family Martyniaceae (1) and *Phaseolus vulgaris* L. in the family Leguminosae (7). In 1934 Grant (2) presented a radically different view of the situation; he infected 29 of 121 tested species in 14 different families among 40 that he investigated. His recognition of wider susceptibility depended on the use of more effective methods of inoculation than had been available for most earlier studies and on observation of symptoms other than systemic mottling. It seemed to the writer that an even larger proportion of tested species might be found susceptible to infection with strains of tobacco-mosaic virus if other methods of determining susceptibility were used to supplement the usual method of observing symptoms after inoculation.

On this account, 2 auxiliary tests were applied to plants of 73 species of herbaceous dicotyledons. Most, but not all, species were subjected to both tests. One of these tests was dependent on quantitative measurement of virus developed at the site of inoculation, the other was dependent on conspicuous local lesions. In the first test, a green-mottling strain of tobacco-mosaic virus was used; in the second, a yellow-mottling strain of the same virus. To date 63 per cent of the 73 species have proved susceptible. These include some that have long been supposed to be immune.

It is the purpose of this paper to present the data derived from the 2 sets of tests. The observations are not primarily of importance as indicating the wide range of disease caused by tobacco-mosaic virus, since many of the newly demonstrated hosts localize the virus and are little injured by its presence. The results are considered significant rather because they show a close relationship between susceptibility of plants and taxonomic affinities. Perhaps there is a corresponding orderly distribution in nature of some substances or conditions important for increase of tobacco-mosaic virus. The failure of the virus thus far to multiply *in vitro* lends interest to any conceptions with regard to factors allowing or preventing its increase.

MATERIALS AND METHODS

Plants were secured for testing by transplanting a wide variety of dicotyledons found growing as weeds in greenhouses, fields, and woods in the vicinity of Princeton, N. J. Susceptibility tests were performed only on young plants, in a greenhouse held at a temperature of about 22° C. (rarely below 21° C. or above 27° C.). The plants were later grown on maturity for identification of species. Aid in identification was kindly given by members of the staff of The New York Botanical Garden.

The first type of test consisted of quantitative measurement to detect increase of virus at the site of inoculation. For this test all plants were inoculated with typical tobacco-mosaic virus (distorting strain of tobacco-mosaic virus, 4, p. 847; 5, p. 897). Inoculation consisted of rubbing 3 or more leaves of each plant with a cheesecloth pad saturated with expressed juice of mosaic plants of Turkish tobacco (*Nicotiana tabacum* L.), diluted with about 50 parts of water. The rubbed area on the leaves was then further inoculated by making at least 100 pin punctures through the residual inoculum with No. 00 insect pins bound together in a fascicle of 5. Generally, only one plant of each species was tested. The rubbing treatment constituted an inoculation sufficient under the conditions of the tests to produce about 150 primary infections in a comparable leaf area of *N. langsdorffii* Weinm., used as a control on infectivity of inoculum. Pin-puncture inoculation was not thought to be so effective as rubbing inoculation, but it served to mark the inoculated leaves for later identification and furnished a safeguard against the possibility that some species, not susceptible to infection by rubbing, might yet prove susceptible by pin-puncture inoculation.

Ten to 12 days after inoculation, the marked leaves from each plant were separately wrapped in cheesecloth, and crushed. Diluted and undiluted expressed juices from each sample were used to inoculate 5 leaves of a plant of *Nicotiana langsdorffii.* A species was considered susceptible when undiluted juice expressed from inoculated leaves induced the production of more than 10 necrotic primary lesions on the leaves of *N. langsdorffii.* Nearly all the susceptible species produced concentrations of virus sufficient to give far more than this minimum number. A few may have been incorrectly classified by adherence to this arbitrary limit, but it was necessary to use some standard. This seemed a suitable requirement, since experience has shown that residual virus on leaves thus inoculated gives less than this number of lesions. Moreover, of the 3 species giving small but significant numbers of lesions in the subinoculation experiments, 2 showed susceptibility also when the second type of test was applied.

All but 2 of the species not shown to be susceptible by this test for virus increase, together with many of the species known to be susceptible, were subjected to the second type of test. This involved inoculation with a yellow-mottling strain (572 D1) derived recently from the distorting strain of tobacco-mosaic virus. This strain produced yellow-mosaic symptoms in tobacco and in many other susceptible species. The symptoms induced by it have been represented in an earlier paper (5, Fig. 1, a), as typical of those produced by highly invasive yellow-mottling strains of tobacco-mosaic virus. The typical tobacco-mosaic virus used in the first test rarely produced visible local lesions, but the yellow-mottling strain often gave evidence of its increase in the inoculated tissues by production of yellow lesions at the site of inoculation, even in plants in which it did not produce systemic infection. It was necessary to avoid large amounts of virus in the inoculum when typical tobacco-mosaic virus was used, lest residual inoculum interfere with

tests for increase of virus. The yellow-mosaic strain, on the other hand, could be applied without dilution, since no subinoculation test was to be made. Through its use, a few species, judged by the tests for virus increase to be doubtfully susceptible, or insusceptible, were shown to be susceptible.

A large part of this work was completed before the description by Rawlins and Tompkins (8) of their useful method of inoculation with carborundum. A few additional trials were made by this method, but they failed to infect species previously found insusceptible. Future development of better methods for inoculation and for recognizing infection may finally show a larger proportion of species to be capable of supporting increase of tobacco-mosaic virus, but present methods seem to have demonstrated the existence of a naturally insusceptible group.

RESULTS OF EXPERIMENTS

In the accompanying table, the tested species are arranged by families in the order in which they appear in Gray's New Manual of Botany (3). Susceptibility is indicated by the letter S in the 5th column wherever the results of subinoculation tests for virus increase (3rd column) or results of inoculation with the yellow-mottling strain of virus (4th column) justify such a classification. Some of these species were later tested for virus in uninoculated leaves at the tops of the plants. A record of the number of lesions produced by this subinoculation is given with that for the inoculated leaf. It appears after a semicolon and a small letter s, signifying systemic infection. Thus, in the table, *Polygonum hydropiper 550*; *s, 0* indicates a plant with a localized infection; in this species much virus was detected in the inoculated leaf after 10 days, but none in uninoculated leaves at the top of the same plant after a month. On the other hand, *Hedeoma pulegioides 950*; *s, 930* signifies a plant with systemic infection; in this species much virus was detected in juice expressed from each source.

The distribution of susceptible and insusceptible species in the table suggested the existence of 4 natural groups: 1st, a largely susceptible group, from Polygonaceae to Cruciferae, of 9 families, with 22 susceptible and 2 insusceptible species; 2nd, an almost entirely insusceptible group, from Rosaceae to Umbelliferae, of 11 families, with 2 susceptible and 16 insusceptible species; 3rd, an entirely susceptible group, from Verbenaceae to Rubiaceae, of 5 families, with 13 susceptible and no insusceptible species; and 4th, a group only partly susceptible, comprising the families Lobeliaceae and Compositae, with 9 susceptible and 9 insusceptible species.

Consideration of the tested families from the viewpoint of their probable relationships as represented by Mez and Ziegenspeck (6) discloses associations of susceptible species that are still more striking than those shown in the linear arrangement of the table. The Königsberger Stammbaum is an evolutionary diagram, constructed by the aid of serological tests that determine affinities of plant species on the basis of common antigenic constituents. A reduced representation, following the plan of the Königsberg diagram, is shown in figure 1, A. Only families tested in the present study are included;

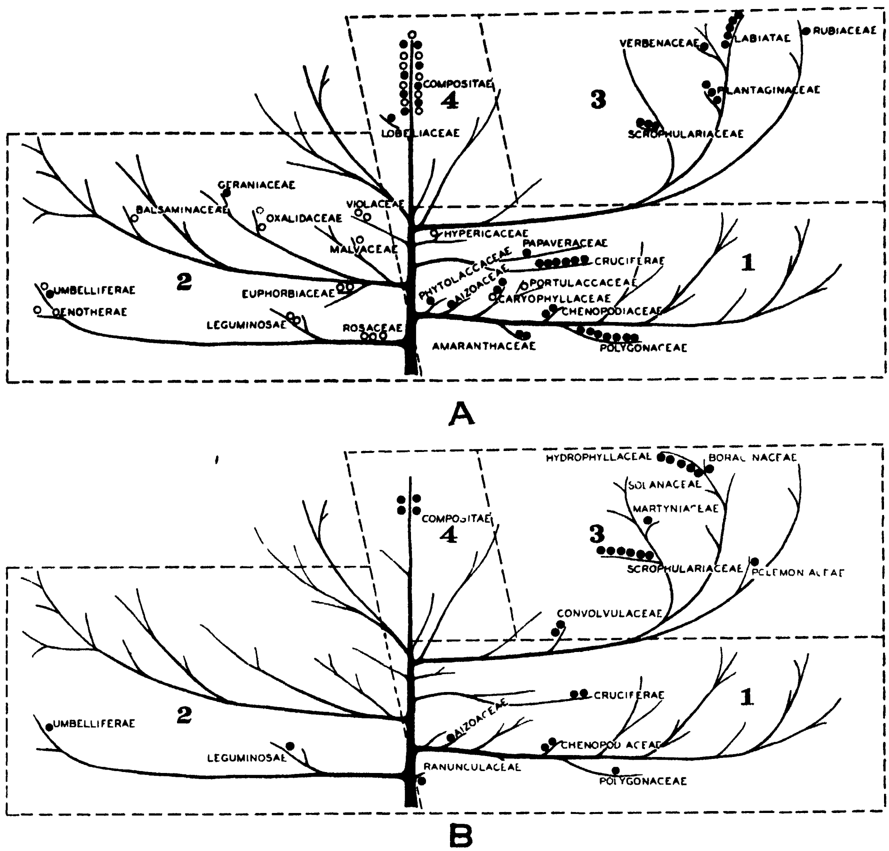

FIG. 1. Taxonomic relationships of plants susceptible to infection by tobacco-mosaic virus. A. Families of tested dicotyledonous plants, diagrammatically arranged according to the plan of the Königsberger Stammbaum of Mez and Ziegenspeck (6). Species tested by inoculation with tobacco mosaic virus are indicated by circles; black circles indicate susceptible species; white circles, insusceptible species. B. Similar diagram, showing distribution of species found susceptible by Grant in 1934 (2).

species are indicated by circles (black for susceptible, and white for insusceptible species), and the 4 groups are separated by broken lines. The 1st group of families, mentioned previously as consisting largely of susceptible species, comprises 3 branches on the right of the diagram. The 2nd group of families, consisting mostly of insusceptible species, constitutes 3 branches on the left side. The 3rd group of families, comprising only susceptible species, constitutes a single great branch on the right; this branch carries the Solanaceae, not included in this study, but known to contain many susceptible species. The 4th group, of mixed susceptibility, represents the top of the main stem of the diagram. The 4 groups in the table and the corresponding groups in the diagram differ in but one species, *Hypericum boreale* (Britton) Bicknell of the Hypericaceae, which appears in the 2nd group of the table but in group 1 of the diagram.

The diagrammatic representation shows more clearly than the linear arrangement of the table that susceptible species occur almost entirely in

TABLE 1.—*Arrangement of tested species of plants by families, with results of experiments performed for detection of increase of the distorting strain, and for detection of symptoms induced by a yellow-mosaic strain (572 D1) of tobacco-mosaic virus*

Family	Species	Lesions on subinoculation; distorting strain	Symptoms observed yellow-mosaic strain; 572 D1	Susceptibility
Polygonaceae	*Rumex crispus* L.	550	Yellow primary lesions; red peripherally	S
	" *acetosella* L.	350	None	S
	Polygonum aviculare L.	600	Large elongated yellow primary lesions	S
	" *erectum* L.	180	Elongated yellow primary lesions	S
	" *persicaria* L.	290; s, 2	None	S
	" *hydropiper* L.	550; s, 0	None	S
	" *convolvulus* L.	570	Yellow primary lesions, elongated along veins	S
Chenopodiaceae	*Chenopodium ambrosioides* L.	900	Necrotic primary lesions	S
	" *album* L.	250	Small yellow primary lesions, becoming necrotic	S
Amaranthaceae	*Amaranthus hybridus* L.	6	Yellow primary lesions	S
	" *graecizans* L.	31	Yellow primary lesions, becoming semi-necrotic	S
Phytolaccaceae	*Phytolacca decandra* L.	2	Yellow primary lesions on mature leaves only	S
Aizoaceae	*Mollugo verticillata* L.	500	Yellow primary lesions, becoming necrotic	S
Caryophyllaceae	*Stellaria media* (L.) Cyrill.	0	Few yellow primary lesions	S
	Cerastium vulgatum L.	700	Zonate yellow primary lesions, becoming necrotic	S
	Lychnis alba Mill.	5; s, 0	None	
Portulacaceae	*Portulaca oleracea* L.	0	None	
Papaveraceae	*Chelidonium majus* L.	570; s, 0	Diffuse yellow primary lesions	S

TABLE 1.—(*Continued*)

Family	Species	Lesions on subinoculation; distorting strain	Symptoms observed yellow-mosaic strain; semicolor 572 D1	Suscepti-bility
Cruciferae	*Lepidium ruderale* L.	330; s, 90	Not tested	S
	" *campestre* (L.) R. Br.	740; s, 0	Yellow primary lesions extending down veins	S
	Capsella bursa-pastoris (L.) Medic.	600	Yellow primary lesions	S
	Sisymbrium officinale (L.) Scop.	65	Multizonate yellow primary lesions	S
	" *thalianum* (L.) J. Gay	525	Not tested	S
	Radicula palustris (L.) Moench.	180; s, 0	None	S
Rosaceae	*Potentilla arguta* Pursh	0; s, 0	None	
	" *monspeliensis* L.	0; s, 0	None	
	Geum canadense Jacq.	0; s, 0	None	
Leguminosae	*Trifolium repens* L.	0	None	
	Medicago sativa L.	4; s, 0	None	...
Oxalidaceae	*Oxalis stricta* L.	0	None	
	" *corniculatus* L.	0	None	
Geraniaceae	*Geranium carolinianum* L.	39	Yellow primary lesions and systemic yellow mottling	S
Euphorbiaceae	*Acalypha virginica* L.	3	None	
	Euphorbia maculata L.	0; s, 0	None	
Balsaminaceae	*Impatiens biflora* Walt.	9; s, 0	None	
Malvaceae	*Malva rotundifolia* L.	0; s, 0	None	
Hypericaceae	*Hypericum boreale* (Britton) Bicknell	0; s, 0	None	
Violaceae	*Viola papilionacea* Pursh	0	None	
	" *sagittata* Ait.	0; s, 0	None	
Onagraceae	*Circaea lutetiana* L.	0; s, 0	None	
Umbelliferae	*Sanicula canadensis* L.	0	None	
	Daucus carota L.	450	None	S
Verbenaceae	*Verbena urticaefolia* L.	44; s, 0	None	S
Labiatae	*Prunella vulgaris* L.	275; s, 0	Yellow primary lesions	S
	Lamium amplexicaule L.	450	Yellow primary lesions	S
	Hedeoma pulegioides (L.) Pers.	950; s, 930	Systemic yellow mottling	S
	Lycopus virginicus L.	135	None	S
	" *rubellus* Moench.	86; s, 0	None	S

TABLE 1.—(*Concluded*)

Family	Species	Lesions on subinoculation; distorting strain	Symptoms observed yellow-mosaic strain; 572 D1	Susceptibility
Scrophulariaceae	*Linaria vulgaris* Hill	400	Zonate yellow primary lesions	S
	Veronica officinalis L.	0	Few zonate yellowish green primary lesions	S
	" *peregrina* L.	56	None	S
Plantaginaceae	*Plantago major* L.	400	Necrotic primary lesions	S
	" *rugelii* Dcne.	81; s, 0	Yellow primary lesions, becoming necrotic	S
	" *lanceolata* L.	100	Yellow primary lesions, becoming semi-necrotic	S
Rubiaceae	*Galium triflorum* Michx.	19; s, 0	None	S
Lobeliaceae	*Lobelia inflata* L.	65	None	S
Compositae	*Solidago rugosa* Mill.	0; s, 0	None	
	" *graminifolia* (L.) Salisb.	0; s, 0	None	
	Aster ericoides L.	0; s, 0	Not tested	
	" *multiflorus* Ait.	0; s, 0	Not tested	
	" *lateriflorus* (L.) Britton	0; s, 0	None	
	" *dumosus* L.	390; s, 0	Not tested	S
	Erigeron annuus (L.) Pers.	255; s, 0	Yellow primary lesions	S
	" *canadensis* L.	1	Yellow lesions, becoming necrotic	S
	Ambrosia artemisiifolia L.	17	Few yellow primary lesions	S
	" *trifida* L.	13; s, 0	Few yellow primary lesions	S
	Rudbeckia hirta L.	0; s, 0	None	
	Helianthus tuberosus L.	0; s, 0	None	
	Bidens frondosa L.	430; s, 0	Yellow primary lesions	S
	Galinsoga parviflora Cav.	270	Yellow primary lesions, elongated along veins	S
	Cirsium arvense (L.) Scop.	5; s, 0	None	
	Taraxacum officinale Weber	1	None	
	Hieracium scabrum Michx.	0; s, 0	Few yellow primary lesions	S

closely associated families (at the right of the diagram), and that insusceptible species are also definitely grouped together (at the left of the diagram). The Compositae, shown at the top of the diagram, are known to have affinities with families at each side; it is not surprising, therefore, that they show susceptibility in some species and not in others.

To summarize the data presented in table 1 and figure 1, A, it may be stated that 46 of the 73 tested species proved susceptible. One of the 46 species (*Daucus carota* L.. wild carrot) has been reported previously as being susceptible (2); Grant infected a cultivated form of this species.

For comparison with the distribution of the 46 species here found susceptible, the 29 non-solanaceous hosts originally reported by Grant (2) are shown in figure 1, B; only 2 of these, *Daucus carota* and *Phaseolus vulgaris*, fall into the 2nd group of families shown in the table and diagram. Grant found all 5 tested species of the Hydrophyllaceae to be susceptible; this family was not represented in the writer's tests, but it, like the largely susceptible family Solanaceae, belongs to the 3rd group, in which all tested species proved susceptible.

DISCUSSION

Substances and conditions requisite for increase of tobacco-mosaic virus are evidently more widespread in herbaceous dicotyledons than has been previously recognized, 63 per cent of the tested species in all families proving susceptible. If the species of plants in the 11 families referred to as constituting the largely insusceptible group 2 are excluded from consideration, 80 per cent of the remaining species are susceptible. If the moderately susceptible group 4 also is eliminated, 95 per cent of the remaining species (35 of 37) are susceptible; these species are distributed in 14 related families. Recognition of so large a proportion of susceptible species as here reported is dependent on the use of 2 tests for virus increase *at the site of inoculation.* Exclusive use of tests for *systemic spread* of virus, including measurement of virus increase and observation of symptoms, permits the recognition of far fewer susceptible species.

Localization of virus within the inoculated leaf was found to be a very common phenomenon among the tested species. It is probable that systemic invasion by tobacco-mosaic virus occurs in only a minority of susceptible species among flowering plants in general.

It is often desirable to obtain virus from pairs of species not closely related to each other, since unrelated hosts are not likely to possess identical antigenic compounds or proteins. If hosts are selected from families in group 3 of the table on the one hand, and from families in groups 1 or 4 on the other hand, numerous pairs of susceptible, but distantly related, plants may be obtained. Some of the chosen species may localize the virus, but objection to their use need not be raised, even though traces of inoculum from the original host plant may be considered undesirable in extracted juice. The difficulty can be avoided by a series of transfers from leaf to

leaf in the new species, to eliminate all previous constituents of the original host plant, except the tobacco-mosaic virus, itself.

SUMMARY

Among 73 tested species of herbaceous dicotyledons, about two thirds (46) proved susceptible to infection with tobacco-mosaic virus. The criteria of susceptibility were detection of increase of virus in inoculated leaves by quantitative subinoculation tests and observation of symptoms at site of inoculation. A correlation seemed to exist between accepted taxonomic classification and susceptibility, since almost all of the tested species in one group of 11 families apparently lacked ability to support increase of tobacco-mosaic virus, whereas 95 per cent of the species in another group of 14 families were shown to have this ability.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
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ADDITIONAL STUDIES CONCERNING THE RUST OF IRIS, PUCCINIA IRIDIS[1]

E. B. MAINS

(Accepted for publication Sept. 28, 1937)

In a previous paper[2] 2 races of *Puccinia iridis* (DC.) Walh. were distinguished by the reactions of strains of *Iris fulva* Ker.-Gawl. and *Iris foliosa* Mackenzie and Bush. None of the varieties of bearded *Iris* of the *Pogoniris* section was successfully infected by these 2 races. In 1928 rust was reported on German iris in the vicinity of San Diego, California, but an attempt to obtain material for study was unsuccessful. In 1934 a report was received from M. W. Gardner that rust was severe on certain bearded varieties in a varietal planting of the University of California at Berkeley, Calif. Dr. Gardner kindly furnished material for study, including a selected set of varieties of *Iris*, and generously has allowed the use of his notes concerning varietal differences in the planting at Berkeley.

During the winters of 1934–1935 and 1935–1936 this rust was studied in a greenhouse on a number of varieties and species of *Iris*. At the same time a comparison was made with the race *septentrionalis* (Tables 1–3).

The rust from Berkeley evidently is a distinct race. The name *Puccinia iridis* race *californica* is proposed for it. Table 4 gives a comparison of the 3 races now recognized on differentiating strains of *Iris*.

As shown in table 1, most of the varieties of bearded *Iris* were highly resistant to the race *californica*. Dr. Gardner's notes show that the same was true in the varietal planting at Berkeley. His notes may be summarized as follows. The varieties Ivory Coast, Leonato, San Rafael, Santa Fe, and *I. longipetala* were severely rusted. Blue and Gold, Colusa, Lady Foster, Magnifica, Pale Moonlight, Pluie d'Or, and Santa Barbara were moderately rusted. California Blue, Don Quixote, Fortuna, Mme. Cherie, Oruga, Padre, Purissima, and Shining Water varied from slight to moderate infection. New Albion, Arkansas, Dolly Madison, Elgante, Frieda Mohr, Gold Top, Modoc, San Luis Rey, Sierra Blue, Sitka, Tulsa, Wm. Mohr, Yosemite Falls, *I. douglasiana*, and *I. microsiphon* were slightly rusted. Asia, Easter Morn, El Capitan, Glowing Embers, Pacific, Pink Lass, Senorita, Uncle Remus, and *I. fimbriata* showed only a trace of rust. Ambassadeur, Blue Gown, Bravura, Bronze Beacon, Dream, Estrallon, Fro, Georgia, Hidalgo, Jacqueline Guillot, J. B. Dumas, Monterey, Pastel Shades, Rayo de Sol, Rialgar, Rosado, Rose Mitchell, Small Yellow, and Sun Dew developed no rust.

As shown in table 1, all of the varieties of bearded *Iris* were highly resistant to the race *septentrionalis*. *I. bucharica* was very resistant to

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 634. The inoculation experiments were made possible through facilities furnished by the University Botanical Gardens.

[2] Mains, E. B. Host specialization in the rust of *Iris*, *Puccinia iridis*. Amer. Jour. Bot. 21: 23–33. 1934.

californica and very susceptible to *septentrionalis* (Table 2). Other species and varieties did not show a pronounced difference in reaction to the 2 races (Tables 2 and 3).

TABLE 1.—*Comparison of the reaction of bearded varieties of Iris to races of californica and septentrionalis of Puccinia iridis*

Variety	Reaction to race[a]		Variety	Reaction to race[a]	
	californica[b]	*septentrionalis*[c]		*californica*[b]	*septentrionalis*[c]
Afterglow	0		Monsignor	3	0
Alcazar	0	0	Monterey[d]	0	0
Ambassadeur	0	0	Mother of Pearl	0	0
Asia	0		Mrs. Neubronner	0	0
Aurea	0	0	Mrs. H. Darwin	0	0
Autumn King	0	0	Olive White	0	0
Autumn Queen	0	0	Oriflamme	0	0
Ballerine	0	0	Oruga[d]	2–3	0
Bruno	0	0	Padre[d]	1	0
Caprice	0	0	Pallida Dalmatica	0	0
Celeste	0	0	Parisiana	0	0
Crusader	0	0	Pluie d'Or	1	0
Elegante[d]	2	0	Powhatan	0	0
Estrallon[d]	0	0	Prairie Gold	0	0
Fairy	0	0	Princess Beatrice	0	0
Flavescens	0	0	Prospero	0	0
Florentina Alba	0–1	0	Purple King	0	0
Georges Tribolet	1	0	Quaker Lady	0	0
Georgia	0	0	Queen Caterina	0	0
Golden Glory	0	0	Queen of May	0	0
Golden Promise	0	0	Rhein Nixe	0	0
Gypsy Queen	0	0	Rialgar[d]	0	0
Hidalgo[d]	0	0	San Luis Rey[d]	1	0
Indian Chief	0	0	San Rafael[d]	2–3	0
Iris King	0	0	Santa Barbara[d]	2	0
Isoline	2	0	Santa Fé[d]	4	0
Jacquesiana	0	0	Sarpedon	1	0
Jean Siret	1	0	Seminole	0	0
Juniata	0	0	Shekinah	0	0
Kochii	0–1	0	Sherwin Wright	0	0
Leonata[d]	0–1	0	Souvenir de Mme. Gaudichau	1–2	0
Lent A. Williamson	0	0	Susan Bliss	1–2	0
Lohengrin	0	0	Tom Tit	1–2	0
Mme. Chereau	2	0	Vesper Gold	0	0
Magnifica	2–3	0	White Knight	0	0
Mary Garden	0	0			

[a] 0, highly resistant, no uredinia; 1, very resistant, few small uredinia; 2, moderately resistant; 3, moderately susceptible; 4, very susceptible; t, trace of rust.
[b] Results obtained with rust from Berkeley, California. Collected by M. W. Gardner.
[c] Summary of results obtained with rust collected on *Iris spuria* at Ann Arbor, Mich., and *I. versicolor* at Trout Lake, Mich., by the writer and on *I. missouriensis* at Pullman, Wash., by G. W. Fischer.
[d] Varieties received from the varietal planting of the University of California through M. W. Gardner.

Susceptible varieties of *Iris* are not limited to any one section of the genus. Both susceptible and resistant strains occur in such diverse groups as the *Apogon* and *Xiphium* sections (Tables 2 and 3). As a group the *Xiphium* section is one of the most susceptible. Several species contain both resistant and susceptible strains. In *I. spuria*, a very variable species, 2

TABLE 2.—*Comparison of reactions of species of Iris to races californica and septentrionalis of Puccinia iridis*

Species of *Iris*	Reaction to race *californica*	Reaction to race *septentrionalis*
Apogon Section:[a]		
Iris douglasiana Herb.	0	0
I. ensata Thunb.	0	0
I. foliosa Mackenzie and Bush (165)[b]	0	0
I. fulva Ker.-Gawl. (32)[b]	0–1	0
I. fulva (164)[b]	0	0
Dorothea K. Williamson (*I. fulva* × *I. foliosa*)110[b]	0	0
I. hexagona Walter		0
I. pseudacorus L.	0	0
I. macrosiphon Torrey		0
I. missouriensis Nuttall		4
I. purdyi Eastwood		0
I. spuria L. (10463)[b]	4	4
I. spuria (6752)[b]	3	4
I. spuria (7097)[b]		1–2
I. spuria (12683)[b]	1	0
I. unguicularis Poiret	0	0
I. tenax Douglas	0	0
I. versicolor L.	0	0
I. chrysophoenicia Small[c]	0	0
I. elephantina Small[c]	0	0
I. giganticoerulae Small[c]	0	0
I. thomasii Small[c]	0	0
I. tyriana Small[c]	0	0
Pardanthopsis Section:		
I. dichotoma Pallas	2–3	3
Evansia Section:		
I. tectorum Maxim.	0	0
Regelia Section:		
I. hoogiana Dykes	4	4
I. stolonifera Maxim.		4
Regeliacyclus Hybrid Charon	4	
Pogocyclus Hybrid William Mohr	3	
Pogoniris Section:		
I. chamaeiris Bertolini	0–2	0
I. mesopotamica Dykes	1–2	0
I. pumila L.	0	0
Juno Section:		
I. bucharica Foster	1	4
Reticulata Section:		
I. histrio Reichenb.		4
I. reticulata M. Bieb.	2	0–1

[a] Classification of W. R. Dykes. The Genus *Iris*. Cambridge University Press. 1913.
[b] Numbers refer to special strains of the species.
[c] Species received from J. K. Small.

strains were susceptible, one moderately resistant, and one highly resistant. Similar results have previously been obtained with this species for the race *septentrionalis* and *australis*. Also, in previous studies, similar variations were found in *I. virginica* to both *septentrionalis* and *australis*. Varieties of Spanish and Dutch iris (*I. xiphium* and hybrids) show a similar situation to the races *californica* and *septentrionalis* (Table 3). Only one strain each of most of the other species was studied. It is probable that a study of a wider range of strains would show a similar situation in a number of other species. In marked contrast to this is the uniform, high susceptibility of varieties of English iris, *I. xiphioides* (Table 3).

Iris rust in this country has been largely confined to wild species. The discovery of rust on bearded horticultural varieties raises the question whether it may be of importance on cultivated forms. Although the race

TABLE 3.—*Comparison of the reactions of varieties belonging to the Xiphium Section of the genus Iris to races californica and septentrionalis*[a] *of Puccinia iridis*

Variety	Reaction to race		Variety	Reaction to race	
	californica	*septentrionalis*		*californica*	*septentrionalis*
Iris xiphium and hybrids (Spanish and Dutch)			Queen Wilhelmina	2	1
			Rembrandt		3
			S. Roombout	1	3
A. Bloemand	4		S. Van Rujdard	4	
Abraham Storck	t		Theresa Schwartz	1	1
Adrian Backer	2	2	Theodore de Bock	2	2
Bell Chinoise		0–1	Van Everdinger	0–1	2
Cajanus	0–1	0[b]	Wedgewood	1	0–1
Celestial	1		White Excelsior	3	3–4
David Bliss	t	0	W. Verschuur	1	
David Haring	4	4	W. Zuiderveld	4	
Dirk Dalens	0		Yellow Queen	3	2–3
Dr. Haring	4		*I. xiphioides* (English)		
E. B. Garnier	2	2			
Frans Hals	4		Almona	4	
Golden Bronze	4		Cornelia	4	4
Golden Glory	3	3	Grant	4	4
Hobbema	2		Herman	4	4
Huchtenberg	4	4	Mauve Queen	4	4
Imperator	0	0	Mont Blanc	4	4
King of Blues	0	0	Perle des Jardins		4
King of Whites	4	4	Prince Albert	4	4
Leonardo da Vinci	4		Prince of Wales		4
Poggenbeeck	4	3–4	Queen of the Blues	4	4
Prince Henry	3	2–3	Raphael	4	
Queen Emma		4	Sweet Lavender	4	4

[a] Frank P. McWhorter kindly supplied a number of the varieties for this study.
[b] This variety was reported as susceptible (Am. Jour. Bot. 21: 29, 1934). Apparently the material studied was incorrectly named.

californica severely infects some bearded varieties, most are highly resistant. If susceptible varieties should, however, be used extensively in breeding new varieties, the rust might become an important factor for growers of this group. It would be interesting to know the ancestry of the susceptible varieties. It may be that susceptibility was introduced from some other section, possibly through *Pogocyclus* hybrids.

Some breeders are giving considerable attention to several of the wild species, and are selecting and naming special strains for garden plantings. There is a possibility that some of these may prove susceptible to one or more of the rust races. This is specially true for those species of the *Iris spuria* type.

TABLE 4.—*Reaction of differential strains of Iris to three races of Puccinia iridis*

Iris strain	Reaction to race		
	californica	*septentrionalis*	*australis*
Iris fulva (32)	0–1	0–1	4
I. fulva (164)	0	0–1	4
I. foliosa (165)	0	0	
Leonato	4	0	
Santa Fe	4	0	

In the *Xiphium* section the rust might occasionally become important for growers if a source of inoculum should occur on wild species during the growing season near plantings. In the midwest, however, the bulbose varieties usually mature before much rust has developed on wild species.

SUMMARY

A rust of bearded *Iris* at Berkeley, California, is an unnamed race for which the name *Puccinia iridis* race *californica* is proposed (Table 4).

Most of the varieties of bearded *Iris* are highly resistant to race *californica*, and all those studied were highly resistant to race *septentrionalis* (Table 1).

All the varieties studied of English *Iris* were very susceptible to races *californica* and *septentrionalis* (Table 3).

Among the Spanish and Dutch *Irises* the varieties Cajanus, David Bliss, Imperator, King of Blues, and Wedgewood were outstanding for resistance to both *californica* and *septentrionalis* (Table 3).

PHYTOPATHOLOGICAL NOTES

Blueberry Galls Produced by the Fungus Phomopsis.—Galls on the stems of cultivated blueberry plants have in the past been attributed to several agencies, including insects, winter injury, and particularly the crown-gall organism. From investigations at this station, however, it was found that the most common type of blueberry gall is caused by a fungus. The galls occur at the crown and along the stem. At first small dark slits appear in the stem, from which lighter colored nodular growths emerge, eventually protruding 5 mm. to 3 cm. or more (Fig. 1, A and B). They resemble much the overgrowths caused by the crown-gall organism. When older, the outgrowths become dark colored. The following year more galls may appear on the stem in late July, or August, and in time kill the stem. These galls develop and spread rather slowly and several years may elapse between the appearance of the first gall and the death of the stem. The disease occurs principally in those regions where the cultivated blueberries are grown, *i.e.*, Massachusetts, New Jersey, Oregon, and Michigan. The Cabot and Pioneer varieties, so far as known, are the ones that are most susceptible to the disease. Because these outgrowths were found to be spreading in some of the localities where blueberry culture was commercially important, it became urgent to learn definitely the cause of the disease.

Platings on beef agar made from young galls gave an organism thought at first to be crown gall, but inoculations into blueberry stems and other plants susceptible to crown gall made with these fresh isolations failed repeatedly. These attempts were made both in the fall and the following spring. Later, the organism was identified as *Bacillus radiobacter*, which resembles strikingly the crown-gall organism. No other bacterial organism appeared on the plates in sufficient numbers to be considered a possible patho-

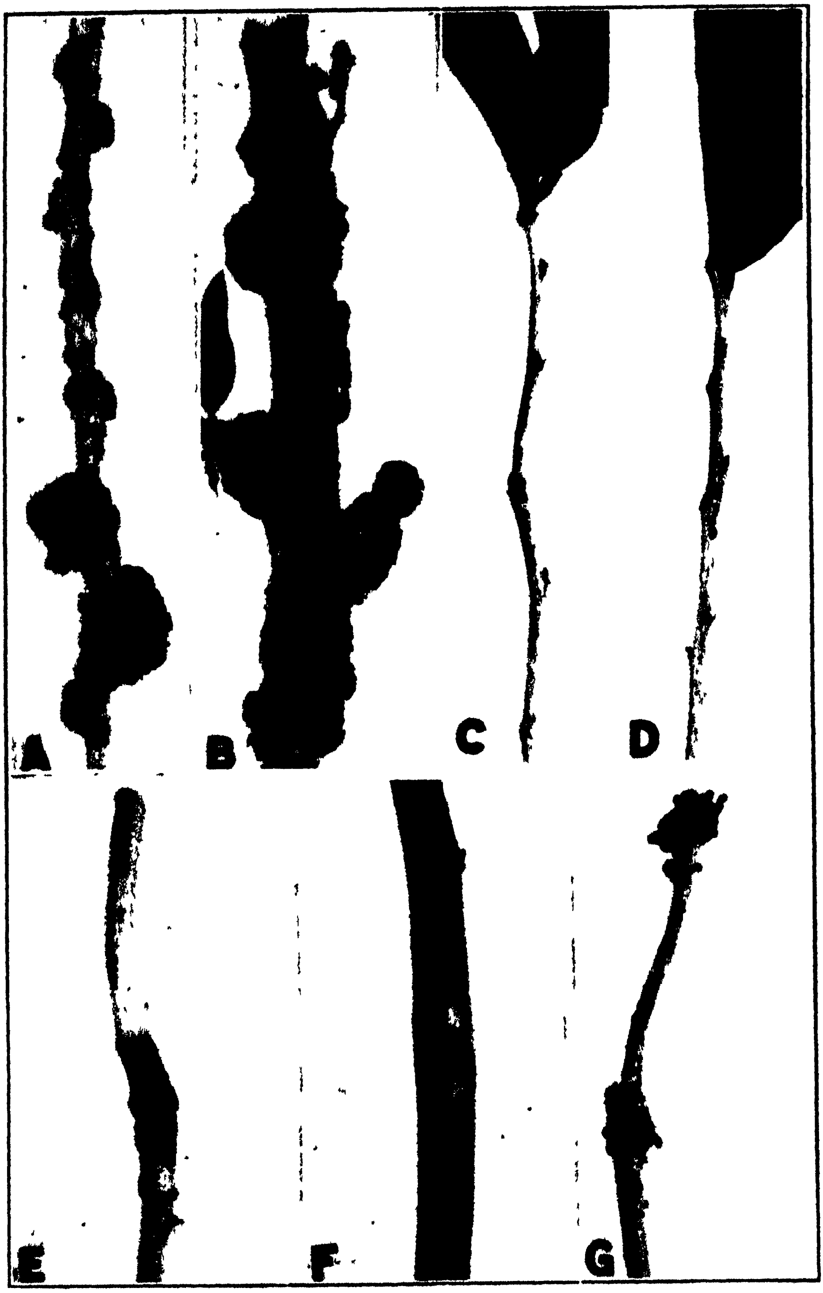

FIG. 1. A and B. Galls on naturally infected cultivated blueberry. A. From New Jersey. B. From Massachusetts. C and D. Inoculations into cultivated blueberry stems, with two strains of *Bacterium tumefaciens*, on 5-12-37. Photographed on 9-21-37. Negative results. E. Inoculation into cultivated blueberry plant, with New Jersey isolation of *Phomopsis*, on 5-12-37. Photographed on 9-21-37. F. Inoculation into cultivated blueberry, with Massachusetts isolation of *Phomopsis*, on 5-12-37. Photographed on 9-21-37. G. Inoculation into *Viburnum opulus* with Massachusetts *Phomopsis*, on 5-16-37. Photographed on 8-24-37. All × 1.

gen. Further evidence that the organism isolated was not *Bacterium tumefaciens* was obtained by inoculating with various strains of *Bact. tumefaciens* into young shoots of cultivated blueberry plants to see if one or more might be infectious (Fig. 1, C and D). The peach, raspberry, hop, and dahlia strains were used. There was some evidence that one of the strains of *Bact. tumefaciens* caused a canker-like reaction in tender blueberry stems but it proved to be of limited extent, as the lesion soon healed over.

In the study of the galls no fungus fruiting-bodies were found on any of those examined and very little fungus mycelium could be detected by microscopic examination of young galls. Nodular galls whose soft tissues necrose or die quickly offer a ready foothold for various saprophytes, so that isolation of the pathogen is difficult. This difficulty, however, was overcome by rather long sterilization of the surface of the galls in mercuric chloride 1–1000, then making and later comparing hundreds of tube cultures. By this method many pure cultures of the fungus *Phomopsis* from various lots of material were obtained. Its pathogenicity was proved by inoculation into cultivated blueberry plants under greenhouse conditions favoring rapid growth. With moist conditions while the fungus was getting established, followed by rather dry conditions, over 50 per cent of the inoculations with both Massachusetts and New Jersey isolations produced outgrowths (Fig. 1, E and F). *Phomopsis* was isolated also from galls obtained from Oregon blueberry plants. It should be noted that the *Phomopsis* isolated from galls on blueberry is not the same one that produces stem-tip blight of blueberry.

Jasminum nudiflorum and *Viburnum opulus*, whose tissues do not harden so readily as those of the blueberry, were also used as host plants for the blueberry *Phomopsis*, and on these plants galls formed more quickly and, in some instances, roots were formed in addition to galls (Fig. 1, G). Root formation was not observed on blueberry stems. Both *Jasminum* and *Viburnum* are attacked by a gall disease somewhat resembling the blueberry gall in appearance and in both cases produced by a *Phomopsis*.[1,2]

A few serious outbreaks of this disease have occurred, but these have been traced to carelessness in rooting cuttings from diseased plants. If reasonable care is taken in propagating blueberries from disease-free plants, there is not much danger of spreading the disease. By persistent effort, the disease can be eradicated as has been done in public parks in the case of the gall disease of *Viburnum* by removing and burning all infected parts in the autumn, when the overgrowths can be readily seen. This method of eradication can be followed with the blueberry.—NELLIE A. BROWN, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Elsinoë on Lemon Fruits from Paraguay.—What is regarded now as *Elsinoë australis* Bitancourt and Jenkins, the cause of sweet orange fruit

1 Brown, Nellie A. A fungus gall on viburnum mistaken for crown gall. Phytopath. 24: 1119–1120. 1934.

2 Privet and jasmine galls produced by a species of *Phomopsis*. Phytopath. 26: 795–799. 1936.

scab, has been found in its conidial (*Sphaceloma*) stage on 2 New York importations of lemon fruits (*Citrus limonia* Osb.) recently received from San Lorenzo, Asunción, Paraguay, the principal citrus-growing dstrict of that country (Fig. 1). The identification was made on the basis of 2 fruits intercepted on May 14 from the first importation (Port of N. Y. 71218) by Inspector G. J. Nicholaïdes and from one fruit taken from

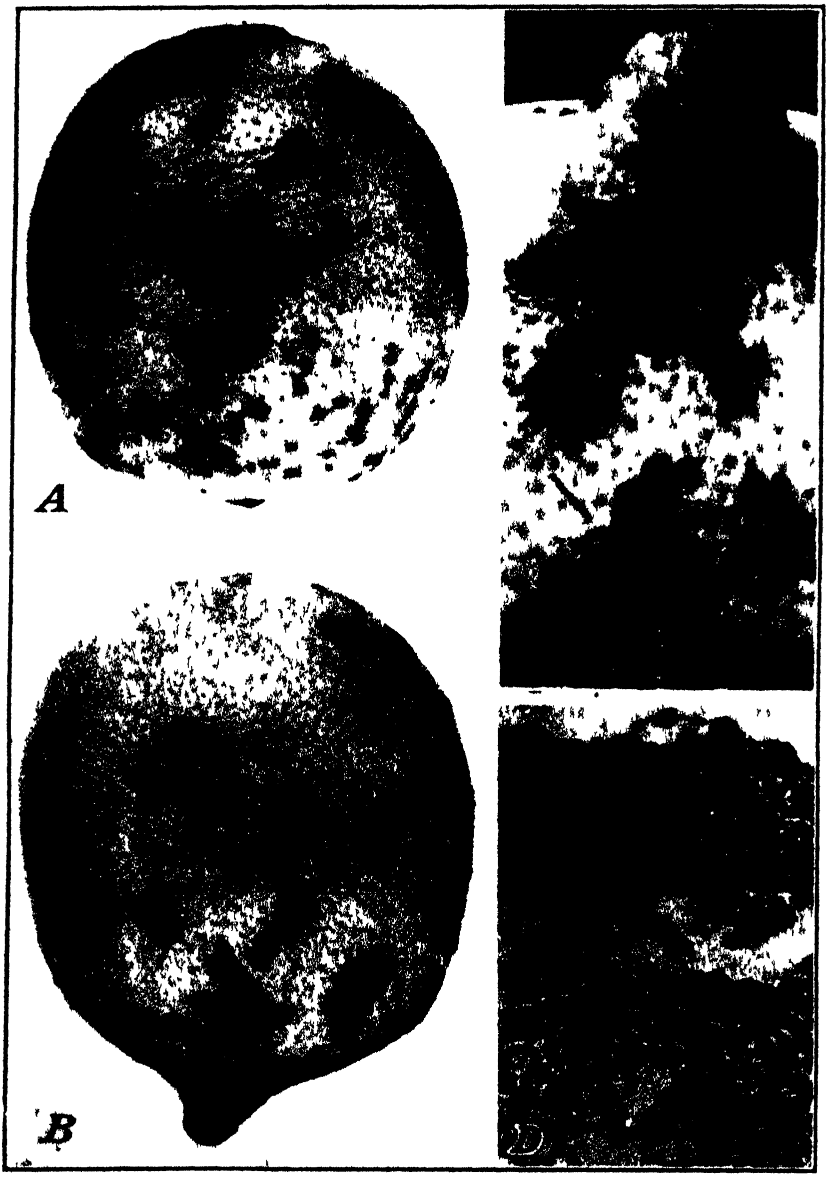

FIG. 1. *A* and *B*. *Elsinoe* on lemons from Paraguay, intercepted at the port of New York, on May 14 (*A*) and May 22 (*B*), 1937. ×1. *C*. Enlargement of lesions from *B*. ×3. *D*. Small acervuli on lesion.

the second importation (Port of N. Y. 71326) and intercepted by Inspectors R. A. Fox and E. Kostal. The severely diseased and exceptionally unattractive appearance of the rind also was noted by C. O. Bratley, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, who saw the importations. The distinctive lesions are chiefly burnt sienna in color. The intercepted fruits were severely affected and in the second shipment the infection was recorded as general. The plant quarantine records show that the only previous importation (July, 1936) was disease-free, as was learned from Max Kisliuk, in charge of Plant Quarantine Inspection at New York.

In their recent study of sweet orange fruit scab, Bitancourt and Jenkins[1] made every effort possible to obtain any available information that might be interpreted as applying to the history and distribution of sweet orange fruit scab in South America. Balansa's classic mycological herbarium specimen of what is now regarded as *Elsinoë australis* on sweet orange from Paraguay (1882), was, of course, known, but not the susceptibility of lemon to attack by what appears to be the same fungus.

The identification of the organism on lemons from Paraguay in May was immediately confirmed by letters from H. S. Fawcett, of the California Station, and A. A. Bitancourt, of the Instituto Biologico, São Paulo, Brazil, telling of their visit to Paraguay in April, and of observing and gathering lemons in the same locality, affected by what they also considered to be *Elsinoë australis.*

This fungus, which causes a scab of sweet orange fruit in South America, has so far not been found in the United States. Consequently, its appearance on lemon fruits imported for sale in our markets constitutes a new and important source of danger to our citrus-growing industry. The fungus was isolated and cultured from the imported fruit and its viability thereby proved. An isolation of the fungus had been made in March, from sour orange (*Citrus aurantium* L.) collected September 1936, in Argentina, by Mr. G. L. Fawcett, of the Estacion Experimental Agricola, at Tucumán, as well as previously (1934) from a sweet orange several weeks after it had been intercepted at New Orleans.—ANNA E. JENKINS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

An Elsinoë causing an Anthracnose on Hicoria pecan.—An *Elsinoë* was discovered on *Hicoria pecan* (Marshall) Britton at Campinas, São Paulo, Brazil, on May 20, 1937, by A. S. Costa and J. M. Salles, Instituto Agronomico de São Paulo, Campinas. Specimens, together with a photomicrograph of the perfect stage, were contributed by Costa. Isolations from ascospores made on May 31 and from acervuli on June 1 yielded slow-growing compact cultures. From Rand's[1] discussion of an imperfect fungus found on pecan in the United States, provisionally iden-

[1] Bitancourt, A. A., and Anna E. Jenkins. Sweet orange fruit scab caused by *Elsinoe australis*. Jour. Agr. Res. [U. S.] 54: 1–18. 1937.

[1] Rand, F. V. Some diseases of pecans. Jour. Agr. Res. [U. S.] 1: 303–337. 1914. (Nursery blight, pp. 305–312.)

FIG. 1. *A. Elsinoe randii* on upper leaf surface of *Hicoria pecan*, São Paulo, May 20, 1937. ×1. *B.* Part of *A*, showing acervuli and ascomata; *C*, reverse of *B*. Both ×5. *D* and *E*. Acervuli: *a*, conidiophore palisades; *b*, underlying stromata; *c*, palisade leaf tissue. *D*, ×250; *E*, ×300. *F*. Section of acervulus (*a*) cultured 24 hours on potato dextrose agar droplet showing the conidia produced, these mostly germinated (*b*); (*c*) palisade leaf tissue. ×200. *G*. Contiguous ascomata: *a*, disrupted epidermis; *b*, palisade leaf tissue; *c*, epithecium; *d*, ascospores. ×500. Photomicrograph contributed by A. S. Costa. *H–K*. *Sphaceloma* on mycological herbarium specimens of pecan leaves from (*H*) Georgia, Oct. 23, 1911, and (*I*, *J*, *K*) Mississippi, Oct. 22, 1928. *I*; *J*, *a*; *K*, *a*, acervuli; *K*, *b*, hyphae; *K*, *c*, leaf palisade; *J*, *b*, and *K*, *d*, probably swollen conidia. *H*, ×1; *I*, ×25; *J*, ×250; *K*, ×350.

tified as *Phyllosticta caryae* Pk., the writers had earlier considered this to be the conidial stage (*Sphaceloma*) of an *Elsinoë*. The new ascomycete from Brazil is the perfect stage of the organism isolated and studied by Rand, or else it is closely related. It is proposed to name it in honor of Doctor Rand, who made a careful study of what is now known to be *Sphaceloma*, growing on pecan in its native home, illustrating cultures and also the effect on leaves. A technical description follows:

Elsinoë randii sp. nov.

Produces cinereous areas on upper leaf surface, sometimes with purplish to black margins, often penetrating leaf as brown to black discolorations; spots round or irregular, .5–5 mm. in diameter, outlining midrib and veins or occupying area between veins of leaflet; ascomata epiphyllous, round or oval, sometimes irregular, pulvinate, 80–200 μ in diameter, dark brown to black, formed intraepidermally, covered with external disrupted epidermal walls and lying directly on palisade layer of host, 50–120 μ thick, with hyaline light brown pseudoparenchyma containing scattered or crowded embedded asci and covered with darker epithecium; asci globose to egg-shape, walls thick, 14–21 μ in diameter, containing 4 to 8 hyaline, 3-septate ascospores, constricted at middle septum, 6–8 μ × 11–16 μ.

Sphaceloma stage: Acervuli epiphyllous, round or irregular, intraepidermal, erumpent-superficial, up to 50–150 μ in diameter, yellow, brown, or black, compact, sometimes almost entirely pseudoparenchymatous with dark crowded conidiophores apparently coalesced, 15–50 μ thick, underlying plectenchyma scanty, hyaline; conidia (in culture) hyaline, continuous or 1-septate, 4–5 μ × 8–15 μ. (Fig. 1, A–G and Fig. 2).

Areas superne cinereas, saepe atro marginatas, inferne discolorentes, in foliis producens; maculis variis, rotundis vel irregularibus, usque 5 mm. in diam., nervisequentibus vel nervis limitatis; ascomatibus epiphyllis, fusco-brunneis, rotundis vel irregularibus, pulvinatis, 50–120 μ crassis; ascis globosis ovatisve, 14–24 μ in diam., 4–8-sporis; ascosporis 3-septatis, 6–8 × 11–16 μ; acervulis epiphyllis, rotundis vel irregularibus, 50–150 μ in diam., 15–50 μ crassis; conidiis (in culturis) continuis vel bicellularibus, 4–5 × 8–15 μ.

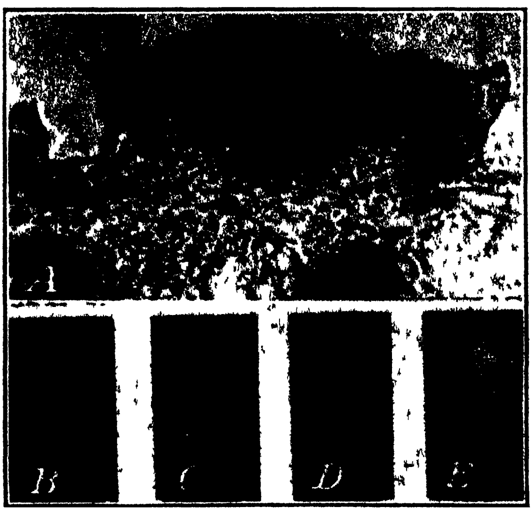

FIG. 2. *Elsinoë randii*. *A*. Acervulus: *a*, conidiophore palisade; *b*, underlying stroma; *c*, ruptured epidermis, São Paulo, May 20, 1937. ×500. *B* and *C*. Original cultures from acervuli. *D* and *E*. From ascospores, all after about 30 days on a modified Thaxter potato-dextrose agar medium. ×1.

On leaves of *Hicoria pecan* (Marshall) Britton, from Campinas, São Paulo, causing pecan anthracnose, May 20, 1937, A. S. Costa and J. M. Salles (type, in Mycological Collections of the Bureau of Plant Industry (No.

72591), Phytopathological Herbaria of Instituto Biologico (No. 2665) and of Instituto Agronomico, (2075), São Paulo). An earlier specimen was collected at Campinas on July 12, 1935, by Costa (Phytopath. Herb. Inst. Agron. No. 1040 and Inst. Biol. No. 2776). Additional specimens examined of the same or a closely related fungus from the United States: Cairo, Ga., Aug. 28 and Oct. 23, 1911, F. V. Rand (Fig. 1, *H*); Washington, D. C., Jan. 1, 1913, F. V. Rand (infection from artificial inoculation); Ovett, Miss., Oct. 22, 1928, C. S. Bentley, communicated by L. E. Miles, October, 1928, to the Plant Disease Survey for identification of the fungus (Fig. 1, *I–K*); Gainesville, Fla., Sept. 29, 1937, W. B. Tisdale.—ANNA E. JENKINS and A. A. BITANCOURT, Bureau of Plant Industry, Washington, D. C, and Instituto Biologico de São Paulo.

Gill Fungi Associated with the Roots of Cereals.[1]—*Pholiota dura* (Bolt.) Fries, *P. praecox* (Pers) Fries, *Naucoria* spp. and a number of small, deli-

FIG. 1. A. *Pholiota praecox* at the base of oat plants (*Avena sativa*). B. *P. dura* at the base of wheat plants (*Triticum aestivum*). Both collected 7 miles southwest of Lebanon, Oregon, May 1, 1934.

cate, undetermined Agaricales have been found attached or associated with the crowns of oats, barley, and wheat in western Oregon (Fig. 1). The white rhizomorphous mycelium of these fungi causes shredding and decay of necrotic and dead leaf sheaths and even at times appears to enter the outer cortical cells of the roots. It has been suggested that these fungi might be parasitic on the cereals.

Several isolations were made during 1930–33 from coarse mycelium attached to wheat and barley plants in the vicinity of Corvallis, Oregon. This mycelium was basidiomycetous in nature, and in one case was attached to immature gill fungi. This latter culture was grown on wheat grain inoculum and introduced into soil, in the greenhouse, in which winter cereals were growing. In all cases over a period of four years wheat, oats, barley, rye, and einkorn were not adversely affected by the fungus; in fact, the inoculated plants were always taller and and stronger than the noninoculated ones.

In 1934 pure-culture isolations were readily obtained from *Pholiota dura* and inoculations again made in the greenhouse. *P. dura* also was nonpathogenic to the cereals and appeared to be mildly beneficial. Data of the investigations are filed in unpublished reports.—RODERICK SPRAGUE, Oregon State College, Corvallis, Oregon.

[1] Coöperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published as Technical Paper No. 273 of the Oregon Agricultural Experiment Station.

TWO HYPHOMYCETES PARASITIC ON OOSPORES OF ROOT-ROTTING OOMYCETES

CHARLES DRECHSLER

(Accepted for publication Sept. 18, 1937)

When sizeable masses of tissue in the larger roots, as also in the basal portions of the stems of plants—more especially of herbaceous plants—are invaded and killed by phycomycetous parasites of the genera *Pythium*, *Phytophthora*, and *Aphanomyces*, they usually come to be permeated by an assortment of adventitious organisms wherein bacteria, fungi, protozoa, and nematodes are represented in greater or smaller measure. As these intrusive organisms cannot well be reached with surface disinfectants, isolation of the oomycetous pathogens primarily responsible for root rots needs to be accomplished in almost all instances through procedure that, except for some discouragement of bacterial multiplication at the beginning, cannot well aim at suppression of such forms of life as may be present (7). Accordingly, after a week or two, the isolation cultures started from the affected tissues often show luxuriant development of microscopic plants and animals, some of which then conveniently reveal on the transparent substratum characteristic parasitic, predacious, or other biotic relationships that undoubtedly prevail also on the natural substrata, but there ordinarily remain concealed from view because of the opaqueness of these materials.

OCCURRENCE, MORPHOLOGY, AND CULTURAL CHARACTERS OF DACTYLELLA SPERMATOPHAGA

Among the organisms thus appearing time after time, year in, year out, in isolation cultures of many species of *Pythium*, is a moniliaceous fungus that I first took for a type of *Fusarium* differing from the general run of forms referred to that genus in its slow growth and in the scanty production of its conidia singly on erect aerial conidiophores. It was noticed early that here and there a branch of the septate mycelium passed narrowly through the wall of an oospore, conidium, or zoosporangium belonging to the root-rotting phycomycete cultured, and, within the invaded reproductive body, terminated in swollen lobulate elements. Though the swollen elements clearly had the appearance of assimilative structures, their presence in itself could not be considered final evidence of parasitism, as the reproductive body might already have been dead at the time it was invaded. For abortive development of sexual apparatus occurs only too frequently in cultures of *Pythium*, often being traceable to such nutritional deficiencies as come into play when, for example, *P. arrhenomanes* Drechsl. (18), *P. periplocum* Drechsl., or *P. sclerotcichum* Drechsl. (10) are grown on unmodified maizemeal agar or on potato agar; the contents of the degenerating oogonia and oospores then being frequently appropriated by such purely saprophytic fungi as may chance to be on hand.

That the moniliaceous form is indeed a parasite became increasingly evident from its constant recurrence in isolation cultures containing oospores or conidia of various species of *Pythium*; from which bodies it manifestly always derived its nourishment. Moreover, it regularly made its appearance in immediate proximity to the pieces of diseased material originally planted on the medium. With the slow extension of its mycelium throughout cultures containing abundant sexual apparatus wholly normal in structural detail, the widening tracts of occupied substratum showed always a steadily increasing proportion of degenerating oospores. This strongly suggested that the hyphomycetous fungus did not wait for the oospores to degenerate spontaneously, but invaded them in their healthy state and actively brought about their destruction.

Closer examination bore out this suggestion. Individual oospores could be found whose central reserve globule, parietal protoplasmic layer and globose or ellipsoidal refringent body still attested a thoroughly normal condition of the protoplast, even after an invading branch had narrowly perforated the enveloping oogonial membrane, had traversed the intervening space, and was perforating the oospore wall itself (Fig. 1, A). From the absence of protoplasmic degeneration previous to invasion, it may be presumed no readily diffusible toxic substance was operative that could be held at all comparable in action to the lethal principle investigated by Weindling (38) and by Weindling and Emerson (39) as a very effective agent in the parasitism of *Trichoderma lignorum* (Tode) Harz on *Rhizoctonia solani* Kühn. Once the invading branch has passed through the oospore wall and into the protoplasmic interior, pronounced changes in internal structure promptly took place. The rather uniformly granular parietal layer and the central reserve globule of homogeneous consistency were both replaced by an irregular arrangement of largish block-like lumps, each of the lumps containing numerous fatty globules of variable sizes. Within this confusion of lumps and granules the progress of invasion by the parasite was always badly obscured. For a period apparently of some duration, it was most difficult to distinguish the haustorial elements from the degenerating protoplasmic materials surrounding them (Fig. 1, H). Following depletion, in large part, of the host protoplasm, the haustorial apparatus could be plainly discerned as a somewhat massive structure composed usually of several swollen lobate elements extending well throughout the cavity of the oospore (Fig. 1, B–G). Once assimilation of the materials within the cavity had been completed, the protoplasmic contents of the haustorial system were withdrawn into the parent hypha, leaving only the empty branching envelope in the collapsing membranous remains of the evacuated sexual apparatus.

Chiefly because the widely distributed damping-off fungus, *Pythium ultimum* Trow, has been obtained more frequently from specimens of diseased plants than any other pathogenic phycomycete, I have observed the moniliaceous fungus most often parasitizing that species in isolation cultures. As might be expected the conidia usually formed in quantity by *P. ultimum* are

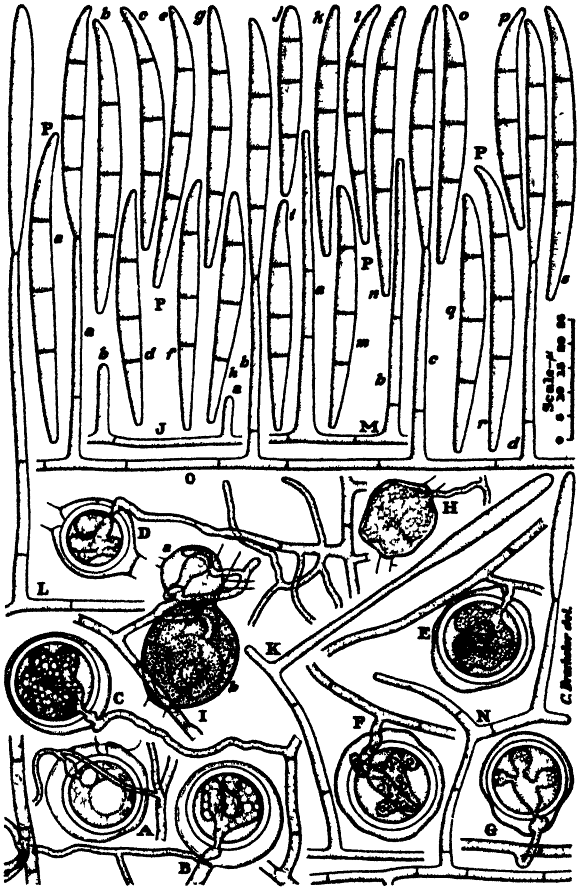

FIG. 1. *Dactylella spermatophaga*; drawn with the aid of a camera lucida to a uniform magnification, from material developed in an isolation culture permeated with *Pythium ultimum*; × 620 throughout. A. Early stage in the invasion of a mature oospore of *P. ultimum* by a hyphal branch of the parasite; the branch having perforated the oogonial wall is penetrating the oospore wall, within which the contents show normal structure—the single large central reserve globule being surrounded by a parietal granular layer that has the single globose refringent body imbedded in it. B–G. Oospores of *P. ultimum* at later stages of invasion by the parasite; as the protoplasmic contents have largely been appropriated, the haustoria are clearly visible as branched lobate structures. H. Conidium of *P. ultimum* soon after invasion by the parasite; the haustorium being concealed in disorganized granular materiel. I, *a*, *b*. Two invaded conidia of *P. ultimum* from which the contents have been largely taken by haustorial elements of the parasite. J. Two young erect conidiophores, *a* and *b*, arising close together from the same mycelial filament. K. A mature conidiophore, which after producing and shedding one conidium, has given rise below its tip to a lateral branch whereon a second conidium is developing. L. Simple and relatively tall conidiophore bearing a young aseptate conidium. M. Two denuded simple conidiophores arising close together from the same superficial hypha. N. A branched conidiophore giving rise to its third conidium. O. A superficial hypha with four erect simple conidiophores, *a–d*, on each of which a mature conidium is borne. P, *a–s*. Mature conidia showing variations in size and shape.

destroyed much in the same manner as are the oospores, and, perhaps, in view of their less substantial walls, with greater readiness (Fig. 1, H; I, *a*, *b*). The parasite has also been observed operative in hundreds of isolation cultures of *P. debaryanum* Hesse, *P. irregulare* Buisman, and *P. mammillatum*

Meurs, always destroying oospores and zoosporangia in its gradual extension through the substratum. Often, too, it has been observed similarly destructive in isolation cultures of *P. butleri* Subr., *P. vexans* de Bary (= *P. complectens* Braun), *P. complens* Fischer, and *P. arrhenomanes;* as well as in isolation cultures of the species I have described elsewhere (8) under the binomials *P. salpingophorum, P. paroecandrum* and *P. oligandrum*. On the other hand, it has never appeared spontaneously in cultures of pythiaceous fungi started from pieces of diseased tissue excised from the interior of such massive plant structures as fruits of the watermelon, *Citrullus vulgaris* Schrad., or as the edible roots of the sweet potato, *Ipomoea batatas* Poir.

After the parasite has been nourished for some time through the destruction of reproductive bodies in isolation cultures of its phycomycetous hosts, it gives rise to scattered conidiophores and conidia. The conidiophores arise as simple erect hyphae (Fig. 1, J, *a, b*) that usually complete their development (Fig. 1, L; M, *a, b*; O, *a–d*) in producing a single elongate-fusoid triseptate conidium (Fig. 1, P, *a–s*), sometimes straight but more often noticeably curved at the tip like the conidia of various species of *Fusarium*. Some conidiophores put forth a branch to produce individually a second conidium (Fig. 1, K); and, occasionally, the first branch gives rise to a second that then forms a third conidium at its tip (Fig. 1, N). Aside from its less abundant conidial septation, the fungus thus shows, at least in mixed bacterium-laden culture, a marked resemblance to *Dactylella passalopaga* Drechsl., a *Fusarium*-like hyphomycetous form capturing and consuming certain species of testaceous rhizopods (17).

The moniliaceous parasite was isolated by removing its conidia from the supporting hyphae directly to tubes of maize-meal agar; the removal being accomplished by means of minute slices of sterile agar held on a flamed platinum spatula, care being taken to avoid contact with the substratum. Growth on maize-meal agar, as also on other artificial media is relatively slow, radial extension usually not exceeding 1 mm. in 24 hours. The vegetative mycelium, which is almost wholly submerged in the substratum, consists of rather delicate, freely branching hyphae in moderately crowded arrangement. When examined with the naked eye it presents for the most part an irregularly cumulous appearance, except in the central portion, where a more uniformly dense texture is usual (Fig. 2, A–D). About a week after a culture has been started, the central portion begins to show a meager greyish or whitish aerial efflorescence that in the course of time is sparingly extended toward the periphery.

Under microscopic inspection this efflorescence is seen to be composed wholly of asexual reproductive apparatus. Successive production of conidia on individual conidiophores following repeated elongation of the fertile axis, encountered only now and then in bacterium-laden isolation cultures, here comes to elaborate expression. In cultures 30 days old, longish sporophoric hyphae may be found weighted down into somewhat procumbent postures by the conidia, often 10 to 20 in number, that are borne on them mostly at

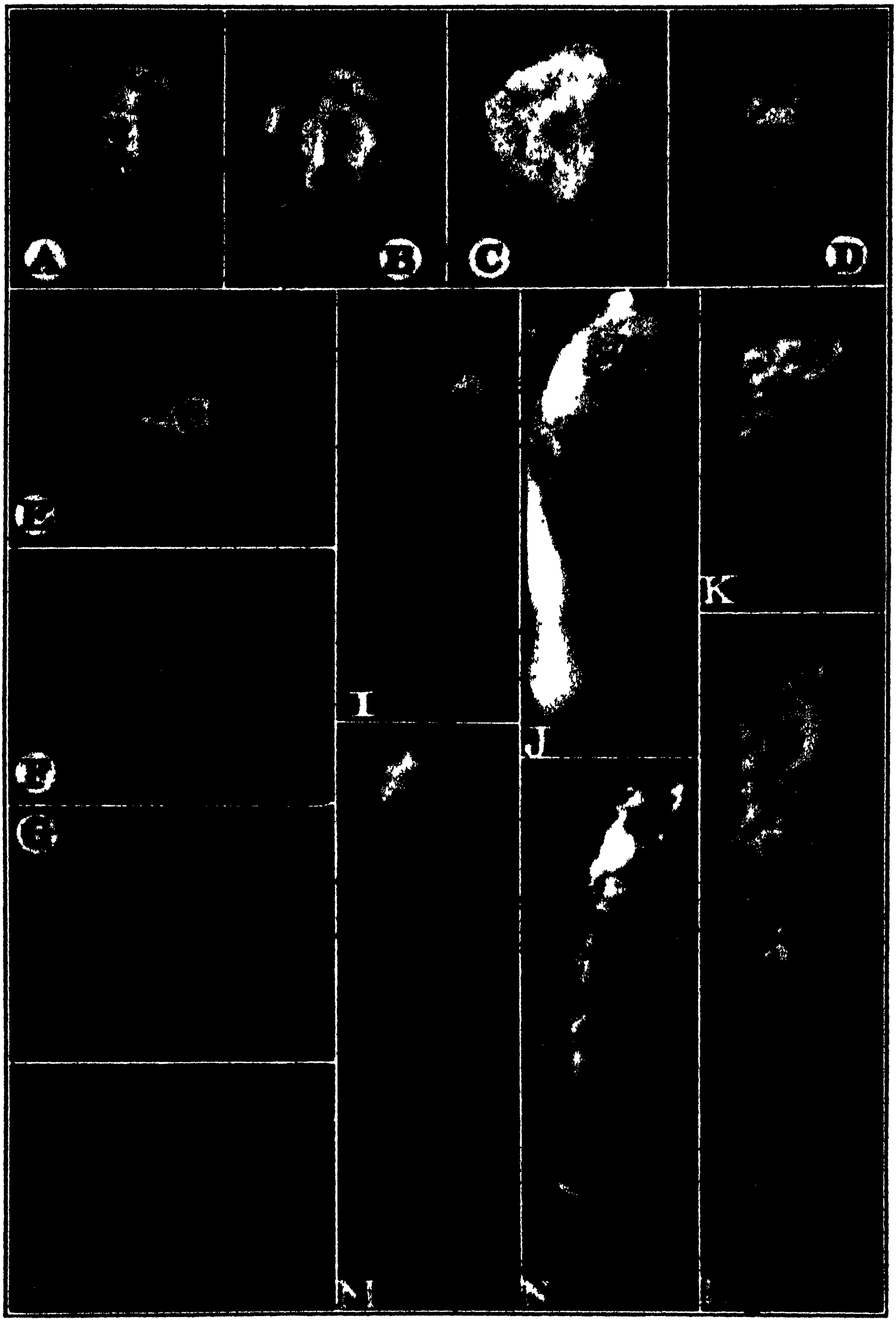

FIG. 2. *Dactylella spermatophaga* photographed from cultures after growing for 13 days at temperatures fluctuating between 25° and 33° C.; all approximately natural size. A–D. Mycelia from maizemeal agar plate cultures, showing rather compact, cumulous texture. E–H. More diffuse mycelia of *D. spermatophaga* resulting from parasitic development in dual culture with *Pythium oedochilum*. I–K. Growth on steamed potato plugs. L–N. Growth on sterilized string-bean pods.

perceptible geniculations and in loose, irregularly spicate arrangement. Sometimes, as often when developing at temperatures between 20° and 25° C., the conidia are conspicuously abnormal in being of small size and irregular shape. Cultures kept at temperatures near 30° C. more often yield conidia similar in shape, size, and septation to those produced in isolation cultures, where, it may be presumed, conditions correspond approximately to those prevailing in nature.

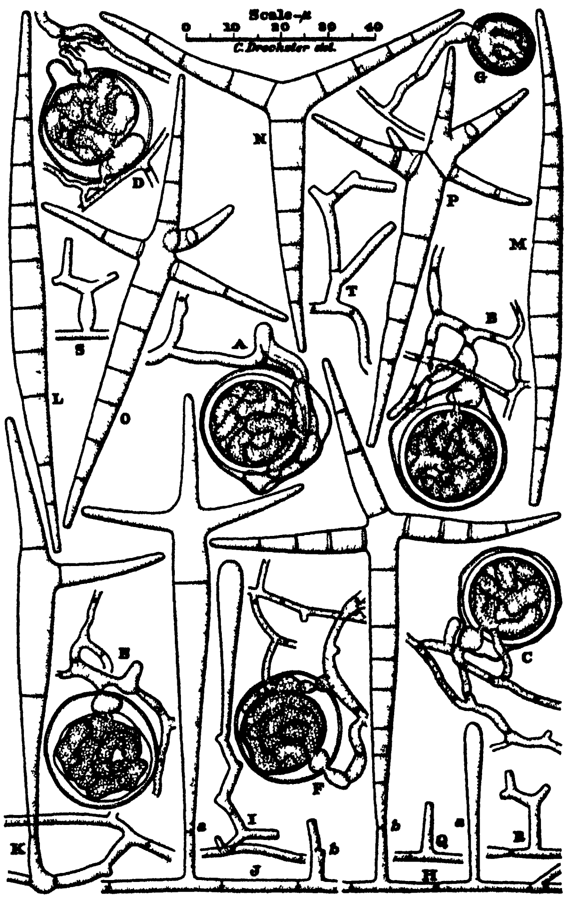

FIG. 3. *Trinacrium subtile*; drawn with the aid of a camera lucida to a uniform magnification, from material developed in an isolation culture permeated by *Pythium butleri*; ×620 throughout. A–F. Oospores of *P. butleri*, each occupied by one or two haustorial systems of *T. subtile*. G. A specimen of the shelled rhizopod *Geococcus vulgaris* invaded and parasitized by a hyphal branch of *T. subtile*. H. A portion of superficial filament bearing a young conidiophorous process, *a*, and also a conidiophore supporting a mature cruciform conidium, *b*. I. A well-developed conidiophore that after producing and shedding 4 conidia in succession, is giving rise to a fifth. J. Portion of superficial hypha with a conidiophore, *a*, bearing its first conidium, which is of cruciform design and immature, as is evident from the single septum; and close by, a second and denuded conidiophore, *b*, that has produced and shed two conidia. K. Portion of mycelium with a conidiophore bearing a somewhat immature conidium. L–P. Conidia, showing variations in dimensions, shape and septation. Q. A denuded simple conidiophore on which one conidium has been produced. R–T. Branched conidiophores whereon 3, 3, and 4 conidia respectively, have been produced.

On steamed potato plugs (Fig. 2, I–K) and on steamed pods of string bean (Fig. 2, L–N) the parasite likewise shows a slow rate of mycelial extension, but develops in more massive quantity. After 20 days the potato plugs reveal usually an irregularly sculptured, somewhat lichenoid growth, which is dull slaty grey where exposed. For the most part, however, it is clothed either sparingly with a sparse downy efflorescence, or slightly more profusely with a whitish velvety covering. On sterilized string-bean pods the aerial growth becomes aggregated here and there in somewhat raised, lichenoid or cartilaginous, waxy or pale flesh-color masses that, like the subjacent growth, may remain partly bare and partly become overspread with effuse or tufted, whitish or dirty greyish, aerial mycelium. In tubes with the bean pod partly immersed in water, a rather substantial cartilaginous, waxy or flesh-colored shelf may develop at the surface of the liquid. When planted in tubes of steamed rice, the fungus remains for some time scarcely perceptible, but, after 3 or 4 weeks, its development is clearly evidenced by a pale creamy coloration, by a matting together of the substratum, as well as by the presence of whitish downy aerial mycelium on the surface. Bright colorations, of the sort resulting from the growth of many of the familiar species of *Fusarium* associated with plant diseases, have never been observed in cultures of the parasite either on steamed rice or on the two other sterilized vegetable substrata. Neither conidia nor any other kind of reproductive bodies were ever produced in cultures employing one or another of the 3 vegetable materials.

OCCURRENCE OF TRINACRIUM SUBTILE RIESS AS A PARASITE ON OOSPORES

A mucedinaceous fungus, differing rather conspicuously from the one so far discussed, appeared in several maize-meal-agar plate cultures started with pieces of decaying tissue excised from the crowns of some potted spinach plants, *Spinacea oleracea* L., at Arlington Experiment Farm, Rosslyn, Virginia. After a protracted period of hot weather in June, 1936, these plants had become wilted one by one, the wilting in each instance having been followed soon by general collapse of the aerial parts, and death. From nearly all of the excised pieces of tissue, mycelium promptly grew out that in due course gave rise to lobulate zoosporangia and sex organs readily recognizable as those of *Pythium butleri*. To this fungus, often actively destructive in our middle latitudes during spells of high summer temperature, the spinach crown rot is manifestly to be attributed.

When the isolation cultures had become 10 days old, 5 among them showed in the agar adjacent to the tissue fragments numerous oospores of *Pythium butleri* being destroyed by a fungus that penetrated the oogonial and oospore walls and developed a swollen lobulate haustorial system within (Fig. 3, A–F), very much after the manner of the form with triseptate *Fusarium*-like conidia. A difference, apparently of somewhat incidental character, came to light in that the invading branch, after perforating the oogonial envelope, often became markedly inflated in the oogonial cavity, before

proceeding with the perforation of the oospore wall. From the original source in each of the 5 plate cultures the fungus continued to spread steadily until 15 days later it was present throughout the medium in the 100-millimeter Petri dishes, and had destroyed the innumerable oospores of *P. butleri* encountered in the course of its extension. In addition a few specimens of the ovoid testaceous rhizopod *Geococcus vulgaris* Francé were found parasitized by the fungus; a lateral hyphal branch evidently having grown through the mouth of each animal and then given rise inside to a number of swollen assimilative branches (Fig. 3, G).

Sporulation of the parasite took place in moderate quantity here and there over the surfaces of the 5 cultures. Though some of the conidia were elongate-fusoid in shape, they clearly exceeded those of the *Fusarium*-like form in dimensions, sometimes attaining as they did, a length of 115 μ and a diameter of 7.5 μ (Fig. 3, L). Instead of regularly showing 3 septa, these larger uniaxial conidia revealed as many as 13 (Fig. 3, M) or even 15 septa. By far the greater number of spores, however, did not consist of uniaxial bodies, but were branched in various ways. A very considerable proportion were bifurcate,—the proximal element, 4- to 8-septate and often between 40 and 60 μ long, terminating in 2 widely divergent elements, mostly up to 40 μ long and containing about 4 septa, to form a rather symmetrically triradiate structure (Fig. 3, N). An almost equally large number were of cruciform design,—bearing generally somewhat closer to the tip than to the base 2 lateral branches nearly at right angles with the main axis (Fig. 3, H, *b*; J, *a*). A smaller number terminated in 4 (Fig. 3, O) or even in 5 (Fig. 3, P) distal elements.

Conidial production was found to begin with the elongation of a fertile erect aerial outgrowth arising from a superficial mycelial filament (Fig. 3, H, *a*). When this outgrowth had attained approximately its definitive size and its definitive condition with respect to presence or absence of branches, a septum was inserted usually between 5 and 20 μ, often about 10 μ, from the base, thereby setting off stalk from young conidium (Fig. 3, J, *a*; K). Maturation of the delimited spores was accompanied by the insertion of usually 10 to 19 partitions, the number varying approximately with the combined length of the main axis and its branches. After a conidium was formed (Fig. 3, Q) the supporting stalk frequently grew out laterally to produce a second on a fertile extension from 5 to 20 μ in length (Fig. 3, J, *b*); repetition of the process yielding simple or variously branched conidiophores, from the successive geniculations of which the development of 3 (Fig. 3, R, S), 4 (Fig. 3, T), or 5 (Fig. 3, I) conidia could be inferred.

With respect to the morphology of its conidial apparatus the fungus agrees rather well with the description and figures of *Trinacrium subtile* published by Fresenius (23) in 1852. While the nearly symmetrically triradiate conidia (23, Pl. 5, Figs. 14, 15) of the design represented in figure 3, N, were apparently held typical of the species by the early mycologist, he included among his illustrations also a figure of a spore with 4 distal elements

(23, Pl. 5, Fig. 16). The limbs of the triradiate conidia were described as containing 3 to 6 septa and as measuring usually about 40 μ in length, though instances of the proximal limb measuring the equivalent of about 72 μ in length were acknowledged. It is perhaps significant that the species was first recorded as overgrowing the acervuli of another fungus, *Stilbospora* sp., and that the relevant figure (23, Pl. 5, Fig. 17) would seem strongly indicative of a parasitic relationship to the spores of the melanconiaceous form.

The literature bearing on *Trinacrium subtile* is relatively meager. Nearly 3 decades after its original description by Riess and Fresenius, Saccardo published additional figures of the species, wherein conidia of the same triradiate design held typical by Riess and Fresenius were shown both detached and in process of development on conidiophores arising directly from an insect egg (35, Tab. 966). Somewhat later, in a brief diagnosis (36) based more especially on his own material, he characterized the spore elements as being 4- to 5-septate, 25 to 30 μ long and 3.5 to 4 μ wide, and the conidiophores as filiform, 20 μ long and 2 μ wide. Oudemans observed *T. subtile* in a vertical strip of *Pleurococcus* on a beech trunk, *Fagus sylvatica* L., in the Netherlands, without being able to make out any evidence of parasitism. He described the conidia as triradiate; the constituent awl-like radial elements, 25 to 40 μ long, being stated in one account (31) to be divided by 3 or 4 septa into 4 or 5 compartments, and in another account (32) to be divided by 2 to 8 cross-walls into 3 to 9 cells. After Wildeman (40) had listed the fungus in a flora of Belgium, Magnus (30) recorded it on decaying *Berberis* twigs within the region embracing Tirol, Vorarlberg, and Liechtenstein; and more recently Rostrup (34) noted its occurrence on dead branches of *Betula* in Denmark.

The rather small measurements given by Saccardo for width of the radial elements in the conidium of *Trinacrium subtile,* suggests that possibly the Italian fungus recorded on insect eggs may have represented a species less robust than the one found destructive to the oospores of *Pythium butleri.* In any case, however, the oospore-destroying parasite conforms closely to the description and figures published by Fresenius; and its conidial septation shows a range of variation in fair consonance with Oudemans' somewhat variant statements regarding this feature. It is accordingly referred, at least provisionally, to the species originally discovered by Riess and adequately described by him in text quoted by Fresenius.

TAXONOMIC POSITION OF TRINACRIUM SUBTILE AND DACTYLELLA SPERMATOPHAGA

The very evident parallelism in parasitic development between the two Hyphomycetes destructive to oospores leaves scarcely any doubt that they are closely related to one another. A presumption of intimate kinship is supported by the resemblance that the uniaxial conidia intermingled with the branching spores of *Trinacrium subtile* bear to the smaller triseptate conidia of the other species, and also by the similarity of the two species with

respect to the production of successive conidia following repeated elongation of the conidiophore. Now, in morphology of conidial apparatus the fungus with triseptate spores shows unmistakable parallelism and evident kinship especially with *Dactylella passalopaga*. Since *D. passalopaga*, as was pointed out earlier, manifestly belongs to an interrelated series of Hyphomycetes including in its known membership various species of *Trichothecium*, *Arthrobotrys*, *Dactylella*, *Dactylaria*, *Tripospormina*, *Pedilospora*, and *Tridentaria* predacious either on nematodes or on rhizopods, it follows that the 2 oospore-destroying forms must also belong in the same natural alliance, despite the generally different biotic relationship pertaining to both alike. For that matter, the divergence in biotic relationships is not so pronounced as to imply mutual exclusiveness in these relationships; reciprocal overlapping coming to light through the occasional parasitism on the one hand, of *T. subtile* on *Geococcus vulgaris*, and through occasional destruction (20, p. 510), on the other hand, of *Pythium* oospores by the nematode-capturing *Dactylella gephyropaga* Drechsl. Pertinent significance, moreover, may well attach to the fact that all known congeners of *Pedilospora dactylopaga*, a form I described (9) as predacious on the shelled rhizopods *Difflugia globulosa* Duj. and *Trinema enchelys* Ehrenb., were reported on other fungi—*P. parasitans* Höhn. (25) being recorded on *Helotium citrinum* (Hedw.) Fr., *P. ramularioides* Bubák (4) on *Bispora pusilla* Sacc., *P. episphaeria* Höhn. (26) on *Nectria cucurbitula* Fr., and *P. zaczewskii* Gizhits'ka (24) on *Acrothecium tenebrosum* Preuss.

Considered from a morphological viewpoint, the 2 oospore-destroying fungi fit acceptably in the predacious series. The short conidiophores of *Trinacrium subtile* invite comparison with the similarly stubby fertile hyphae of the amoeba-capturing *Dactylella tylopaga* Drechsl. (14); its occasional uniaxial conidia suggest the elongated many-septate spores of *D. leptospora* Drechsl. (20); and its more numerous variously branched conidia recall the homologous branched structures of *Pedilospora dactylopaga*, *Tripospormina aphanopaga* Drechsl. (20), and *Tridentaria carnivora* Drechsl. (21). Repeated prolongation of conidiophores with the production of additional conidia, prevails in conspicuous measure among some of the capitate nematode-capturing forms, as notably in *Arthrobotrys superba* Corda, *A. oligospora* Fres., *A. conoides* Drechsl. (20), and *Dactylaria polycephala* Drechsl. (20); yet certainly, under especially favorable conditions, is not wholly absent among the predacious species of *Dactylella*, even though this genus is defined—and with respect to development on natural substrata, properly defined—as producing solitary acrogenous conidia. Indeed, such repeated prolongation is found not altogether rarely, even in bacterium-laden cultures of *D. passalopaga*, with which fungus, as has been stated, the smaller oospore-destroying parasite would seem most intimately allied. This parasite, having apparently not been described hitherto, possibly because its commonplace appearance and its very meager development of reproductive apparatus invite little attention, is accordingly presented as a new species of *Dacty-*

lella; a term meaning "eating seeds" being deemed a suitably descriptive specific name.

Dactylella spermatophaga sp. nov.

Sparsa; mycelium ramosum, hyphis sterilibus hyalinis, .8–4 μ crassis, aliis angustis, flexuosis, filiformibus, parce septatis, aliis latioribus, torulosis, crebro septatis,—quibusvis ramulum tenuem in corpora perdurantia Pythiacearum saepe intrudentibus, haustorium obesum ramosum plerumque plus minusve lobosum intus evolventibus quod protoplasma assumit; hyphis fertilibus hyalinis, erectis, septatis, simplicibus vel interdum parce ramosis, vulgo 35–75 μ altis, basi 2.5–4 μ crassis, sursum leniter attenuatis, apice 1–2 μ crassis, ibi unicum conidium ferentibus, atqui subinde identidem repullulantibus, 10–15 additicia conidia deinceps gerentibus, tum usque 300 vel 500 μ longis et fere aliquid procumbentibus. Conidia hyalina, elongato-fusoidea, recta vel saepe praecipue in parte superiore curvata, basi rotunde truncata, apice rotundata, 35–65 μ (saepius circa 50 μ) longa, 3.8–5.2 μ (saepius circa 4.5 μ) crassa, vulgo triseptata, rarius biseptata vel quadriseptata.

Hyphas, conidia, zoosporangia, oogonia, praecipue oosporas multarum specierum Pythiacearum (magnam partem Pythii et Phytophthorae) interimens, habitat late in terra et in materiis plantarum putrescentibus, prope Beltsville, Maryland et in Arlington, Virginia.

Sparse; mycelium abundantly branched; vegetative hyphae hyaline, .8 to 4 μ wide, some narrow, flexuous, filiform and sparingly septate, others wider, toruloid and more closely septate,—whether of one type or the other, often thrusting a narrow branch into the resting reproductive bodies of Pythiaceae, and then, in each instance, giving rise inside to a rather bulky haustorium consisting of more or less lobulate branches usually 2 to 5 μ wide, yet sometimes as much as 6 or 7 μ in width; conidiophores, hyaline, septate, simple or sometimes somewhat branched, commonly 35 to 75 μ high, 2.5 to 4 μ wide at the base, tapering upward to a width of 1 to 2 μ at the tip, whereon is borne a single conidium, —but when developing under especially favorable conditions, often on repeated elongation giving rise at successive intervals to 10 or 15 additional conidia, thereby attaining lengths of 300 to 500 μ and becoming more or less procumbent. Conidia hyaline, elongate spindle-shaped, roundly truncate at the base, narrowly rounded at the tip, straight, or more especially in the distal portion, curved, 35 to 65 μ (average about 50 μ) long, 3.8 to 5.2 μ (average about 4.5 μ) wide, commonly containing 3, more rarely 2 or 4 cross walls.

Parasitizing hyphae, conidia, zoosporangia, oogonia and more particularly oospores of many species of Pythiaceae (mostly species of *Pythium* and *Phytophthora*), it occurs widely in the soil and in decaying plant remains near Beltsville, Md., and in Arlington, Va.

It must be admitted that with respect to reproductive habit neither *Dactylella spermatophaga*, nor certainly *Trinacrium subtile,* shows much direct resemblance to *Arthrobotrys oligospora* Fres., the species most familiarly exemplifying the predacious hyphomycetes; the connection being traceable only rather indirectly through a number of forms embodying transitional types of conidial apparatus. That the chain of similarities here concerned, though perhaps appearing at first somewhat far-fetched, is not beyond the scope of a well-integrated group, becomes manifest from its parallelism with an analogous train of resemblances extending more particularly through the noncatenulate members of the Zoopagaceae, an unquestionably natural family of conidial phycomycetes destructive to nematodes and rhizopods. Thus, the branched conidium produced on the short conidiophore of *Trinacrium subtile,* shows an engaging analogy to the appendaged and virtually sessile conidium of *Acaulopage tetraceros* Drechsl. (12) or of *A. acanthospora* Drechsl. (22); the elongated conidium and moderately developed conidiophore of *Dactylella spermatophaga* recalls the similarly proportioned homologous structures of *Stylopage rhabdospora* Drechsl. (16); the broadly ellipsoidal or obovoid conidia and tall conidiophores of *Dactylella bembicodes* Drechsl. (20), *Dactylella gephyropaga*, and *Trichothecium polybrochum* Drechsl. (20) are

reminiscent of the conidial apparatus in *S. hadra* Drechsl. (13) and *S. leiohypha* Drechsl. (15); while to extend the correspondence, the monocephalous capitate moniliaceous forms as, for example, *Dactylaria candida* (Nees) Sacc. and *Arthrobotrys dactyloides* Drechsl. (20), show a striking similarity of habit to *S. cephalote* Drechsl. (22).

An alternative disposition of the two oospore-destroying forms is, of course, suggested by the remarkable structural resemblance that the conidia of *Dactylella spermatophaga* bear to the conidia of some species of *Fusarium* —a resemblance that gains in possible import from the known occurrence of many species of *Fusarium* on other fungi (41, pp. 302–305). However, assimilation of *D. spermatophaga* to *Fusarium* encounters a serious obstacle in the failure of the fungus to produce conidia otherwise than terminally on discrete aerial hyphae. Clements and Shear (6, p. 402), it is true, have ventured to subsume under *Fusarium* the genus *Rachisia* represented by a single species, *R. spiralis* Lindner, which, after developing endoparasitically in vinegar eels (*Anguillula aceti* Ehrenb.), produces falcate conidia laterally on external discrete rachiform filaments. Unfortunately, Lindner (28) omitted to state whether cross walls are present or absent in the hyphae and conidia of *R. spiralis*; yet, the rachiform conidiophores pictured by him reveal a most persuasive similarity to those of *Graminella bulbosa* recently described by Léger and Gauthier (27) from the rectal cuticle of *Baetis* larvae. Indeed, the resemblance is so striking that, until further information is obtained, Lindner's remarkable parasite might well be extricated from any association with *Fusarium* and placed, even if only somewhat tentatively, in the Harpellaceae, a singular family of conidial phycomycetes occurring especially on the peritrophic membrane and rectal cuticle of the aquatic larvae of various insects.

DACTYLELLA SPERMATOPHAGA IN DUAL CULTURE WITH VARIOUS OOMYCETES

After *Dactylella spermatophaga* had been obtained in pure culture, the scope of its parasitism was further explored by growing it in dual culture together more especially with different pathogenic oomycetes that had not previously been observed being attacked in isolation cultures. In order that spontaneous degeneration of the host fungi may not be held to have impaired the trustworthiness of observations recorded here, only results will be considered that could be obtained by the use of maize-meal agar media clear enough to permit satisfactory inspection throughout and thereby to make possible the confirmation of normal development in the particular oomycetes under trial. Among the species of *Phytophthora* thus tried out, oospores of undoubtedly normal structure were produced in quantity by *P. cactorum* (Leb. & Cohn) Schroet. and *P. megasperma* Drechsl. After their maturation the oospores of both species were successfully parasitized by *P. spermatophaga,* and that despite the thickness of their protective walls. The clustered globose chlamydospores of *P. cinnamomi* Rands were likewise abundantly

attacked and destroyed, as were also the more solitary globose thick-walled chlamydospores or "resting zoosporangia" of *P. parasitica* Dast.

In dual culture with *Dactylella spermatophaga,* oospores of *Aphanomyces euteiches* Drechsl., a pathogen often responsible for serious root rot of peas (*Pisum sativum* L.) during wet seasons, were freely invaded and exhausted of contents. Neither the thick oogonial membrane characteristic of this water mold, nor the substantial oospore wall, appeared to offer any effective barrier to entrance by the hyphomycetous parasite. Rather often, to be sure, the oospore was reached not by perforation of the oogonial envelope, but by penetration of the antheridial wall, followed by elongation of the infective hypha through the antheridial chamber and down the fertilization tube. This easier mode of invasion is apparently facilitated by the frequent presence of 3, 4, or 5 male organs about an oogonium, by the fairly large size of these organs, and by their retention, sometimes, of granular protoplasm, even at relatively late stages of development.

When *Pythium graminicolum* Subr., *P. dissotocum* Drechsl., and *P. periilum* Drechsl., each isolated (8, 18) from diseased roots of sugar-cane, *Saccharum officinarum* L., were grown in dual culture with *Dactylella spermatophaga,* the numerous oospores of correct internal structure produced by them were parasitized in large numbers. Such parasitism was evident also in dual cultures of *D. spermatophaga* and *P. myriotylum* Drechsl., a species occurring widely in our southeastern States as the cause of a blossom-end decay of watermelon fruits (8), as well as of a cottony rot of the fruits of cucumber, *Cucumis sativus* L., closely resembling "cottony leak" caused by *P. butleri.* With no less success *D. spermatophaga* likewise parasitized the oospores of *P. acanthicum* Drechsl. and *P. periplocum,* the 2 forms with characteristically spiny oogonia that are most frequently found associated with blossom-end rot of watermelon fruits in Maryland, Indiana, and Missouri (8). In dual culture on slightly acidulated maize-meal agar containing considerable maize-meal sediment in suspension, the hyphomycetous parasite abundantly destroyed the oospores of *P. scleroteichum,* one of the species frequently causing in edible sweet-potato roots the marbled decay known as "mottle necrosis."

Among morphological modifications to be observed in the sexual apparatus of oomycetes, few are more formidable in aspect or present more of an appearance suggesting a protective function than the sturdy, often characteristically mammiform protuberance normally besetting the oogonia (8) of *Pythium polymastum* Drechsl. and *P. mastophorum* Drechsl. Yet, *Dactylella spermatophaga,* in mixed culture with these impressive and rather rare representatives of the genus *Pythium,* thrusts its infective branches through the sturdily mamelonated oogonial envelopes without apparent difficulty, to reach the smooth, globose oospores within; the infective branches then, of course, individually perforating the oospore wall and extending a lobulate haustorium through the protoplasmic interior. Equally ineffective for protection against invasion by the hyphomycetous parasite is the prominently

echinate oogonial wall of *P. anandrum* Drechsl., a species remarkable alike for its consistent parthenogenesis and for the *Phytophthora*-like shape of its repeatedly proliferous sporangia (8). The handsome parthenospores of the species are readily invaded and destroyed on their development in dual culture with *D. spermatophaga.*

Further testimony to the efficiency of *Dactylella spermatophaga* in penetrating sturdy host structures is furnished by its more than ordinarily profuse development when grown in dual culture with any one of the 4 closely interrelated forms I described (8) under the names *Pythium helicoides, P. oedochilum, P. polytylum,* and *P. palingenes.* In their asexual reproduction these forms follow the development early set forth by de Bary (1) in the description of his *P. proliferum,* giving rise, as they do on appropriate irrigation, to repeatedly proliferous terminal sporangia that individually discharge their undifferentiated contents through an evacuation tube into a vesicle, there to be fashioned into zoospores (Fig. 4, A, B). Much greater distinctiveness attaches to their sexual apparatus, not only in that the elongated antheridia are applied lengthwise to the oogonium rather than apically, as in most species of *Pythium*, but, also, in that the mature oospore regularly reveals imbedded in its somewhat turbidly granular protoplasmic contents usually about a score of reserve globules and about a half dozen refringent bodies (Fig. 4, F, G), rather than only the single reserve globule and single refringent body more generally present elsewhere among the monospermous oomycetes of the genera *Pythium*, *Phytophthora, Aphanomyces*, and *Plectospira.* Of particular relevance to the present discussion is the fact that the oospore wall in each of the 4 species usually averages about 2.5 μ or more in thickness, besides presenting an appearance of marked induration through its frequently yellow or yellowish coloration. Yet, after penetration of the oogonial envelope, this conspicuously substantial wall is perforated without apparent difficulty by the invading branch of *D. spermatophaga*; extension of a massive lobulate haustorial system throughout the protoplasmic interior then ensuing (Fig. 4, H and K), as in the less stoutly incased oospores of the species more frequently isolated.

As *Pythium oedochilum,* when grown on maize-meal agar, is very dependable in producing—and rather abundantly, too—sexual apparatus of uniformly normal structure, it makes an especially satisfactory host in dual culture with the hyphomycetous parasite. On encountering a mycelium of *Dactylella spermatophaga,* many of its vegetative hyphae are invaded over stretches of variable lengths and locally exhausted of their contents (Fig. 4, C–E). With the appearance later of zoosporangia, these are parasitized (Fig. 5, A) no less successfully than are the hyphae and oospores. Thus richly nourished, the parasite gives rise to its most luxuriant display of simple or somewhat branched conidiophores (Fig. 5, B, C) bearing numerous conidia (Fig. 5, D, *a–n*) comparable in shape and size to those produced in pure culture only under exceptionally favorable conditions. Frequently the conidia anastomose with their supporting hyphae previous to normal dis-

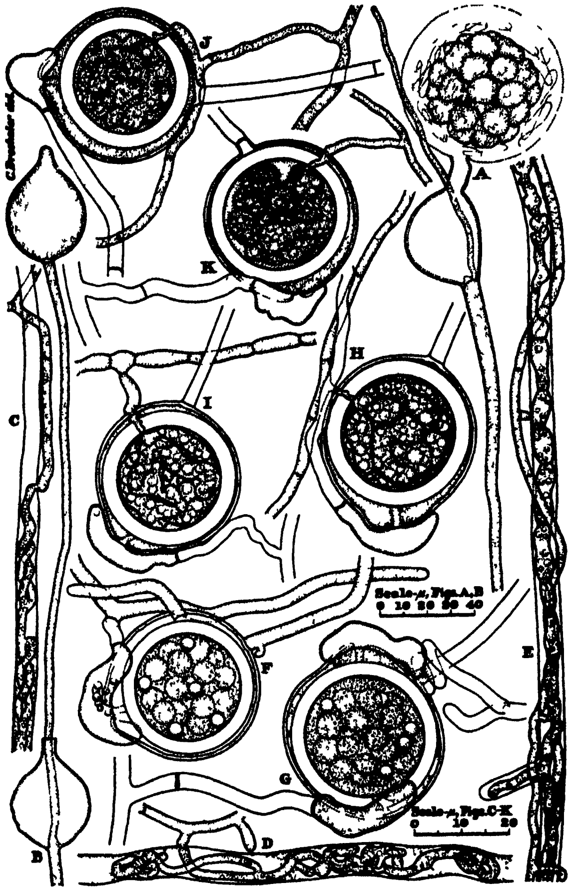

FIG. 4. *Dactylella spermatophaga* and *Pythium oedochilum*; drawn with the aid of a camera lucida, mostly from material developed in dual culture on maizemeal agar. A. A zoosporangium of *P. oedochilum* showing zoospores being formed in a vesicle at the tip of an evacuation tube; the supporting hypha is continuing growth from immediately below the sporangium; × 310. B. Two zoosporangia of *P. oedochilum*, the distal one borne on an axial prolongation of the conidiophore supporting the proximal one; × 310. C–E. Hyphae of *P. oedochilum* parasitized by filaments of *D. spermatophaga;* × 620. F, G. Normal mature sexual apparatus of *P. oedochilum*, showing the internal organization characteristic of the *helicoides* series in *Pythium*—multiple reserve globules and multiple refringent bodies being imbedded in murkily granular protoplasm; spiral involvement of the oogonial stalk by the branch supporting one of the antheridia, in the manner shown in G, occurs infrequently in the species, though regularly present in *P. helicoides;* × 620. H–K. Infected sexual apparatus of *P. oedochilum;* the oospore in each unit having been occupied by a rather massive branching haustorium of *D. spermatophaga*, after successive perforation of the oogonial and oospore walls; × 620.

articulation (Fig. 5, E); or, falling on the substratum, unite vegetatively with one another (Fig. 5, F), sometimes to give rise to secondary conidia (Fig. 5, G). To the naked eye, as might be expected, the parasitic mycelia of *D. spermatophaga* present a more diffuse appearance than mycelia developing in pure culture, rarely showing any suggestion of cumulous texture (Fig. 2, E–H).

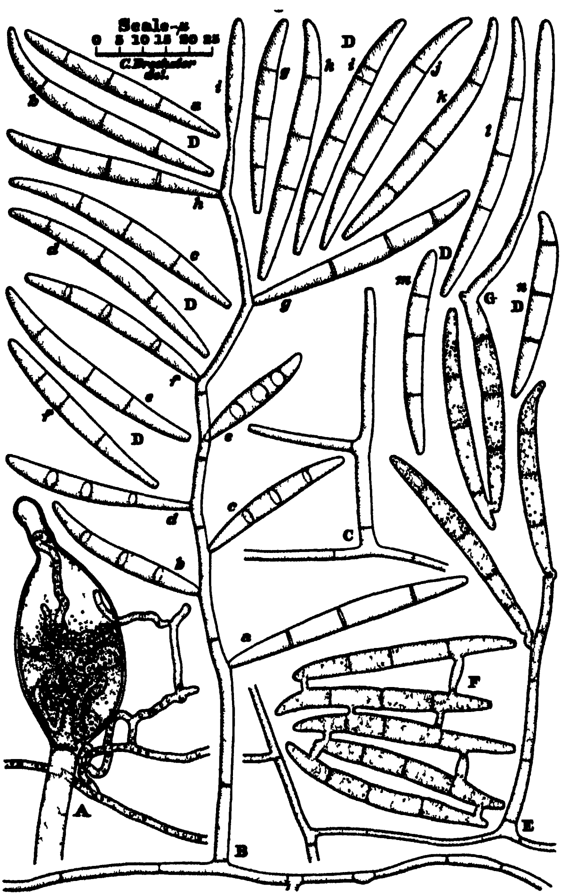

FIG. 5. *Dactylella spermatophaga;* drawn with the aid of a camera lucida to a uniform magnification, from material developed in dual culture with *Pythium oedochilum* on maizemeal agar; × 620 throughout. A. A sporangium of *P. oedochilum* invaded and nearly depleted of protoplasmic contents by the parasite. B. A conidiophore on which have been produced successively 8 conidia, *a–h*; a ninth conidium, *i*, being in course of development at the apex. C. Portion of hypha with a branched conidiophore denuded of its two conidia. D, *a–n*. Conidia, showing variations in size, shape and septation. E. Portion of mycelium with a conidiophore whereon have been produced 2 conidia, both of which have become united vegetatively with the supporting hypha previous to their disarticulation therefrom. F. Five conidia that after falling on the substratum have become united vegetatively through fusion by means of germ tubes. G. Two fallen conidia that have fused vegetatively; from one of them a conidiophore has been produced that is giving rise to a secondary conidium.

THE RÔLE OF DACTYLELLA SPERMATOPHAGA IN THE BIONOMICS OF ROOT-ROTTING OOMYCETES

Though *Dactylella spermatophaga* is unquestionably capable of destroying mycelial filaments of numerous species of *Pythium*, the destruction of vegetative hyphae would seem on the whole far too meager to be of any great importance in the bionomics of the oomycetous hosts. Even the slower-growing species among the phycomycetes subject to attack show rates of mycelial extension from 10 to 15 times faster than that of the parasite, while some hosts, as, for example, *P. debaryanum*, *P. ultimum*, and *P. butleri*,

exceed the linear or radial growth of *D. spermatophaga,* 50 to 75 times. A mycelium extended by a pathogenic oomycete through a sizable portion of root or stem, or through any other rather bulky mass of substratum, would, for the most part, therefore, presumably have ample time to form reproductive bodies before it could be overtaken by the parasite.

Though *Dactylella spermatophaga* may appear rather innocuous in its occasional invasion of vegetative hyphae, as also in its occasional interception of reproductive development, its unceasing destruction of mature oospores and conidia must almost certainly reduce the longevity of its phycomycetous hosts. Evidently widely distributed in the soil, as well as in plant materials decaying moistly in contact with the ground, and freely attacking the most resistant of soil-borne reproductive bodies through which the many root-rotting oomycetes are enabled to persist from season to season, *D. spermatophaga* must be considered an organism with very real potentialities for promoting soil sanitation. Since, in the persistence of oospores, and accordingly also in programs of crop rotation governed thereby, periods of time reckoned in years rather than in days, weeks, or months, are involved, the relatively slow growth of the parasite would seem not seriously incompatible with its efficiency as a hygienic agent. Even in much shorter periods, oospores at first inaccessible to attack because of the massiveness of the phanerogamic structures occupied, become subject to invasion with the disintegration of the substratum. Thus, while the innumerable oospores formed by *Pythium acanthicum* and *P. periplocum* in diseased watermelon fruits may enjoy protection for perhaps a week or two, decomposition of the affected material soon exposes them to the action of the parasite.

OTHER ORGANISMS ANTAGONISTIC TO ROOT-ROTTING OOMYCETES IN ISOLATION CULTURES

Apart from *Trinacrium subtile* and *Dactylella spermatophaga,* oospores of root-rotting phycomycetes in isolation cultures have been found undergoing destruction by amoebae and shelled rhizopods. The various amoebae implicated vary greatly in size. Some are so small that after ingesting a single oospore of *Pythium ultimum* or *P. debaryanum* they appear individually as only a rather thin enveloping layer of protoplasm. Others are large enough to move about briskly after engulfing a dozen oospores of the two damping-off fungi mentioned. Somewhat curiously, the small species of *Amoeba* seem generally much more rapid in killing and digesting oospores devoured by them than are the larger congeneric forms. Oospores have likewise been found ingested by the robust, large-mouthed testaceous rhizopod *Arcella vulgaris* Ehrenb.; or, again, have been observed in process of being exhausted of their degenerating contents by the testaceous rhizopods *Geococcus vulgaris* Francé and *Euglypha denticulata* Brown, following, in each instance, perforation of the oospore wall within an area circumscribed by the mouth of the shelled animal orally applied to it (17, pp. 400–401; 19, p. 244). On the whole, however, the aggregate destruction of oospores seen accom-

plished in isolation cultures by all protozoans together, while sometimes not inconsiderable, seems hardly deserving of comparison with the destruction found achieved by *D. spermatophaga.*

Butler (5, p. 109) directed attention to the somewhat unexpected occurrence of chytridiaceous parasites in soil-inhabiting species of *Pythium*. The pure-culture technique whereby affected portions of root or stem are placed on firm agar culture media, ordinarily gives little encouragement to the development of any organisms able to spread only through locomotion of zoospores. Nevertheless, some slight opportunity for the development of such organisms is often provided by the accumulation of water in a minute pool immediately surrounding the planted decaying material. In the limited area covered by the pool, *Pleolpidium inflatum* Butl., with its large and immensely prolific zoosporangia, can sometimes be found actively parasitizing *Pythium* filaments. Very little free water would seem to suffice for the propagation of *Pleolpidium irregulare* Butl., as this species has been found extending itself successfully in maize-meal agar cultures of *Pythium irregulare* on the surface of which no deposit of liquid was visible. The chytridiaceous parasite was for years maintained in culture free of bacteria and all extraneous organisms by merely transferring it with its fungus host from one tube of firm agar medium to another. It singles out for invasion especially the growing oogonia, which organs are often conveniently identifiable by the characteristic digitations borne on them (5, Pl. 8, Fig. 11); so that its parasitism, if not effecting the destruction of oospores already formed, yet operates, in part, like the parasitism of *Dactylella spermatophaga,* by preventing their formation.

The generally unfavorable effect of bacterial contamination on cultures of most pythiaceous and saprolegniaceous fungi has long been annoyingly familiar both among students of the aquatic microflora and among investigators dealing with plant diseases. Such contamination, being here far more pertinacious than in cultures of most other fungi, usually requires extra effort for its elimination; wherefore, indeed, different special methods of purification have been devised, including some made known in recent years by Brown (3), Volkonsky (37), Machacek (29), Blank and Tiffney (2) and Raper (33). Yet in investigations on root rots and other related plant diseases, discomfiture more serious than any resulting from an unhappy intrusion of bacteria in isolation cultures, has often come in repeated failure to obtain on agar or other artificial medium any growth whatever of the causal parasite concerned; so that in the absence of any isolation culture from which a correct beginning might be made, either no likely pathogenic agent could be uncovered at all, or the disease was (and it may be suspected in many instances still is) wrongly attributed to an adventitious organism. According to the relevant literature, species of *Phytophthora* that in nature develop at relatively high temperatures in the softer succulent tissues of herbaceous or, for that matter, of woody plants, have often been found difficult to get started from pieces of diseased tissue placed on artificial sub-

strata; as have also most disease-producing species of *Aphanomyces* and some root-rotting species of *Pythium* other than the common damping-off forms distinguishable among phycomycetous parasites for their vigorous and extensive extramatrical growth—*P. ultimum, P. debaryanum, P. irregulare, P. mammillatum,* and *P. butleri.*

Microscopic examination of tissues undergoing active invasion by species of oomycetes, not readily started in isolation cultures, has very generally shown the difficulty to lie in a debilitated condition of the parasitic mycelium, evidently attributable, in the main, to the presence of putrefactive bacteria. During warm periods especially, the motile saprogenous bacteria that habitually accompany the phycomycetous filaments in their courses through host tissue, become so active that often many can be seen swarming about the very tips of the invading hyphae, just as under similar conditions they swarm about the tips of superficial hyphae of growing mycelia in isolation cultures. Now, whereas on firm agar media development of bacteria is limited, for some time at least, to the surfaces, so that submerged filaments are protected from the products of putrefaction save as these gradually diffuse through the intervening gel, in invaded plant tissues the multiplying bacteria permeate the substratum throughout, thereby exposing all filaments to direct contact with the saprogenic substances. As a result protoplasmic degeneration becomes evident everywhere except in the youngest hyphae in the forefront of the advancing mycelium. Sometimes, in fact, bacterial development appears to overcome even the filaments in the van of an infection; partly decayed specimens of watermelon fruits, for example, having been found wherein the advance of one or another of the different parasitic *Pythium* species causing blossom-end rot seemed to have been stopped outright. Where remnants of mycelium survive in the marginal portions of a lesion, they nearly always can be induced to grow out conveniently into an agar substratum if first the accumulated products of putrefaction are leached away by bathing the affected tissues in several changes of fresh water, and bacterial development, after the transfer to agar, is discouraged through the removal of all free water previously by pressing the material between pieces of sterilized filter paper (7).

Indeed, within their host plants no less than in artificial culture, the whole behavior of pythiaceous and saprolegniaceous parasites betrays, as a general fundamental character, an incapacity for thriving, or even for long enduring vegetatively in a fouled substratum. With their markedly rapid rate of mycelial extension some species of *Pythium* are enabled to thrust their assimilative hyphae into fresh unfouled masses of plant material, moribund or newly dead, ahead of competing fungi of slower growth, and there to build up a vegetative thallus of sufficient bulk for the development of oospores before the increasing putrefaction brought on by the accompanying bacteria causes protoplasmic degeneration on a serious scale. Despite their sensitiveness to putrefaction such fast-growing species of *Pythium* frequently occur, therefore, in semi-parasitic and saprophytic relationships as well as in truly

parasitic relationships resulting from the invasion of succulent living structures. It may be presumed, on the other hand, that species of *Phytophthora* with their slower mycelial growth are at a great disadvantage in any competitive invasion of plant tissues normally moribund or otherwise deprived of effective resistance; and are consequently, for the most part, limited in their development under natural conditions to tissues newly invaded and freshly killed by them. Their ready saprophytic development in pure culture on a wide variety of artificial and natural substrata, yet always in the absence of putrefactive organisms, must accordingly not be considered inconsistent with their habitual occurrence in nature as parasites. With respect to nutrition, then, the root-rotting oomycetes in general seem comparable to the predacious hyphomycetous fungi and the two related oospore-destroying forms herein discussed; though a notable difference is presented in that, while the hyphomycetous fungi apparently cannot take nourishment from a foul substratum, their mycelia readily endure immersion in putrescent slime. As was pointed out earlier (11) *Pythium anandrum, P. mastophorum,* and *P. polymastum* are appreciably less sensitive to bacterial contamination than the main mass of root-rotting oomycetes; their capacity for producing zoospores in water rather generously laden with bacteria, as well as their somewhat aberrant morphology, perhaps being indicative of special ecological relationships. However, the moderate tolerance of these species to bacterial contamination is not to be confused with the thoroughgoing adaptation to foulness shown by members of the truly saprophilous genus *Pythiogeton*, which grow luxuriantly and produce zoospores abundantly in heavily putrescent slimes.

From some writings on damping off the suggestion is conveyed that addition of stable manure is undesirable, not only because infectious material may be introduced and tender growth favorable to infection encouraged thereby, but also because after such addition the seedling parasites might be able to permeate the soil more readily by developing on the bits of dung distributed through it. Misgivings on the latter score would seem excessive, as certainly the behavior of the pythiaceous damping-off organisms in the laboratory is not such as to justify any fear that they might spread anywhere on a substratum as foul as dung. On the contrary, their behavior suggests that if a seedbed could be kept continuously infiltrated throughout with putrescent substances, development of destructive mycelia from resting conidia and oospores might be effectively prevented. Indeed, it appears within the realm of possibility that where soil sterilization by steam or chemicals is inexpedient, some control of damping off might be achieved by watering seedling flats exclusively with a putrescent infusion not objectionably rich in nitrogenous substances, such as could be conveniently prepared by the retting of herbaceous waste materials.

SUMMARY

Of 2 moniliaceous fungi found vigorously parasitic on oospores of pythiaceous root-rotting organisms, one with mostly triradiate and cruciform

conidia is identified as *Trinacrium subtile*; the other with regularly triseptate *Fusarium*-like conidia is described as a new species under the binomial *Dactylella spermatophaga*. Despite conspicuous differences in conidial design, the 2 hyphomycetous parasites appear closely related to one another. Because of its failure to produce compound conidial apparatus, and in view of its resemblance to *D. passalopaga*, a rhizopod-capturing form, as well as to *D. leptospora*, a nematode-capturing species, *D. spermatophaga*, together with *T. subtile*, is considered related taxonomically to the predacious series of hyphomycetes rather than to the genus *Fusarium*.

Dactylella spermatophaga has been observed to appear spontaneously in numerous isolation cultures started from diseased portions of many host plants obtained in a number of different localities representing collectively a wide variety of environmental conditions. In these cultures it was found destroying oospores of *Pythium arrhenomanes*, *P. butleri*, *P. complens*, *P. debaryanum*, *P. irregulare*, *P. mammillatum*, *P. oligandrum*, *P. paroecandrum*, *P. salpingophorum*, *P. ultimum*, and *P. vexans*. Grown in dual culture with other oomycetes it parasitized abundantly also the oospores of *Pythium acanthicum*, *P. anandrum*, *P. dissotocum*, *P. graminicolum*, *P. helicoides*, *P. mastophorum*, *P. myriotylum*, *P. oedochilum*, *P. palingenes*, *P. periilum*, *P. periplocum*, *P. polymastum*, *P. polytylum*, *P. scleroteichum*, *Phytophthora cactorum*, *Phytophthora megasperma*, and *Aphanomyces euteiches*. Infection is accomplished by perforation successively of the oogonial and oospore walls, followed by development within the oospore of a branched, somewhat lobate, and rather massive haustorium that appropriates the protoplasmic contents.

Because of its evident capacity for widespread destruction of oospores, on which bodies root-rotting oomycetes mainly depend for survival from season to season, *Dactylella spermatophaga* presumably serves as an effective agent in promoting soil sanitation over extended periods of time. A similar rôle in the long-time bionomics of soil-borne oomycetes is played by various amoebae and testaceous rhizopods. Parasitic chytrids, on the other hand, operate not so much to destroy oospores already formed, as they operate to prevent their development; while putrefactive bacteria exert a pervasively debilitating effect on the mycelium of root-rotting oomycetes,—an effect entailing, under natural conditions, the virtual restriction of slow-growing oomycetous forms to strictly parasitic relationships, though permitting the occurrence of faster-growing forms in saprophytic and semiparasitic as well as wholly parasitic relationships.

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MINT ANTHRACNOSE

R. C. BAINES[1]

(Accepted for publication Oct. 19, 1937)

The disease referred to herein as mint anthracnose was brought to the author's attention in 1931, when specimens of infected black peppermint, *Mentha piperita* L. var. *vulgaris* Sole, were received from 2 sections in northern Indiana. While there is no record of the occurrence of the disease prior to 1931, mint growers had observed it a number of years earlier and called it leopard spot. During the past 3 years mint anthracnose has been found in many fields of peppermint and also in a field of spearmint. The degree of infection and amount of injury to the plants varied from very slight to severe. In nearly all cases the disease was more prevalent in fields of old established peppermint than in newly planted fields. However, in 1933, one-third of the plants in a 20-acre field of first-year peppermint were found infected. Nelson[2] briefly described the disease (leopard spot), which was first observed in Michigan in 1935.

DESCRIPTION OF THE DISEASE

Small brown spots are the first symptoms of the disease on young stems and stolons of black peppermint (Fig. 1, A). Later, these areas enlarge and become somewhat oval with a light, or ash colored center surrounded by a dark reddish border (Fig. 1, B). The spots vary from 2 to 6 mm. in diameter and stand out clearly on the green or reddish green background of the stems and stolons. Frequently, numerous lesions coalesce to form large cankered areas, which may entirely encircle the stem and in later stages cause it to crack. Heavily infected young stems and stolons are frequently killed.

The first symptoms of the disease on young leaves are small brown spots, which subsequently enlarge and become visible from both surfaces of the

[1] The writer wishes to express his appreciation to Dr. R. W. Samson and Dr. R. M. Caldwell for suggestions on the preparation of the manuscript.

[2] Nelson, R. Peppermint diseases and their control. Michigan Muck Farmers Assn. Proc. 18: 44–54. 1936.

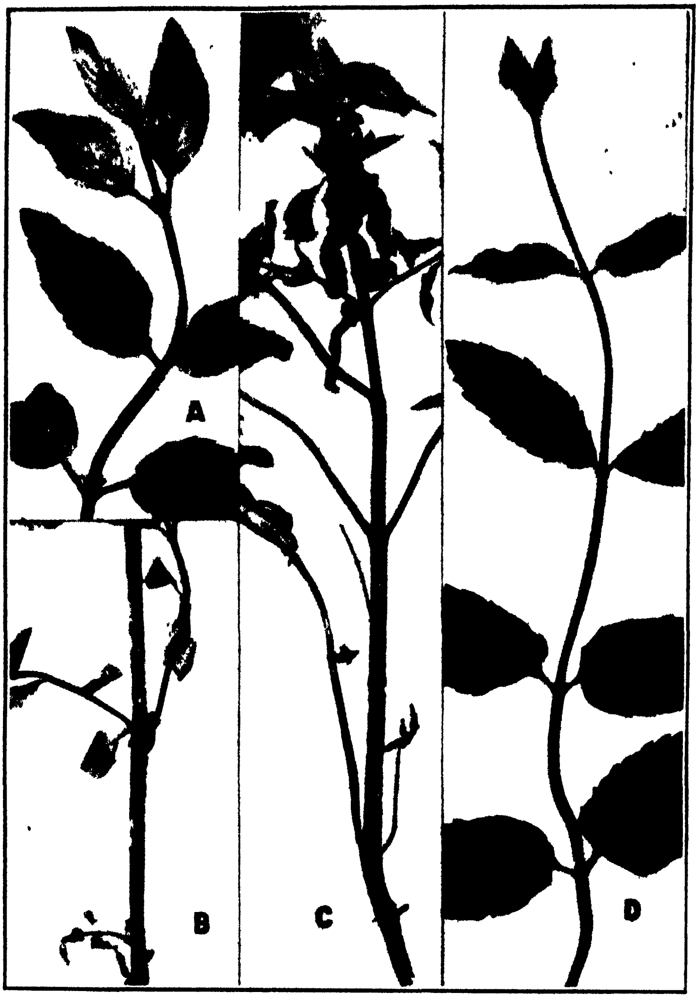

FIG. 1. Symptoms of anthracnose on 2 species of mint. A. Heavy infection on black peppermint shoot; note the constricted stem and yellow terminal leaves. B. Characteristic ash-color lesions with a reddish border on black peppermint stem. C. Lesions on Scotch spearmint stem. D. Lesions on common spearmint stem and leaves 17 days after inoculation. All approximately × 1.

leaf (Fig. 1, A). The spots in later stages are round, approximately 2 mm. in diameter, and light brown with a darker colored margin. Numerous infections on individual leaves may kill large areas of the leaves and cause them to drop prematurely. Frequently, the necrotic areas drop out, giving a shot-hole effect.

The symptoms of the disease on state peppermint, *Mentha piperita* L., white peppermint, *M. piperita* L. var. *officinalis* Sole, and Scotch and common spearmint, *M. spicata* L. (Fig. 1, C and D), are similar to those occurring on black peppermint. On Japanese mint, *M. arvensis* L. var. *piperascens* Malinvaud, the lesions are smaller.

PATHOLOGICAL HISTOLOGY

Stems and leaves of black peppermint with infection in various stages of development were killed in Rawlins' fixative and embedded in paraffin by the n-butyl alcohol procedure outlined by Zirkle.[8] An aqueous solution of anilin blue in picric acid was the most satisfactory stain tried for differentiating the fungus mycelium in the host tissue. A counter stain of orange G was used. The collapsed and dense nature of the contents and walls of the infected cells made it difficult to detect the fungus within the necrotic tissue.

On young stems and leaves the mycelium is, at first, largely superficial or subcuticular. After the epidermal cells are killed, the hyphae penetrate the intercellular spaces of the inner tissues. The cells in contact with and a short distance in advance of the hyphae are killed. Occasionally the infecting hyphae make a limited superficial or subcuticular growth on the leaves before penetrating the underlying tissues. In the section of a young leaf (Fig. 2, B) the infecting hyphae have killed the epidermal cells and then penetrated between the palisade cells. A lesion developing on the ventral side of the leaf usually involves, successively, the epidermis, palisade, spongy parenchyma, and lower epidermis (Fig. 2, A). Frequently the epidermis is sloughed off. Although the spots are limited in size, the continued enlargement of the infected area does not appear to be limited by any visible protective response of the host cells bordering the lesions.

The disease is confined to the epidermis and the cortex of the stems. Affected cells, as in the leaf, die and collapse, to form a slightly depressed lesion (Fig. 2, C). The intercellular mycelium is only sparsely distributed through the necrotic tissues. In the vicinity of the infection, the parenchyma cells of the cortex enlarge, and to a limited extent divide, although no wound phellem is formed. Frequently, a stromatic layer of mycelium is formed on the surface of older lesions from which the conidia are abstricted (Fig. 3, A, B).

ISOLATION AND IDENTIFICATION OF CAUSAL FUNGUS

In the summer of 1933 the causal fungus was isolated from young lesions on the stems and leaves of black peppermint, by the tissue-planting method, and from dilution plates of conidia. The fungus grows slowly and forms a light brown, frequently convoluted type of colony on potato-dextrose agar (Fig. 3, E). In 1936 a fungus similar to that from peppermint was isolated from lesions on the stem of Scotch spearmint. Attempts to isolate the fungus from old lesions were unsuccessful, because of competition of fast-growing secondary organisms in the tissues.

The conidia from cultures are usually slightly larger than those from lesions on the host. Conidia produced on leaf lesions (Fig. 3, C) are hyaline, oblong-elliptical, continuous, sessile on the surface of the superficial, thin stromatic layer and average $6 \times 10\ \mu$ in size with extreme dimensions of

[8] Zirkle, C. The use of n butyl alcohol in dehydrating woody tissue for paraffin embedding. Science (n. s.) 71: 103–104. 1930.

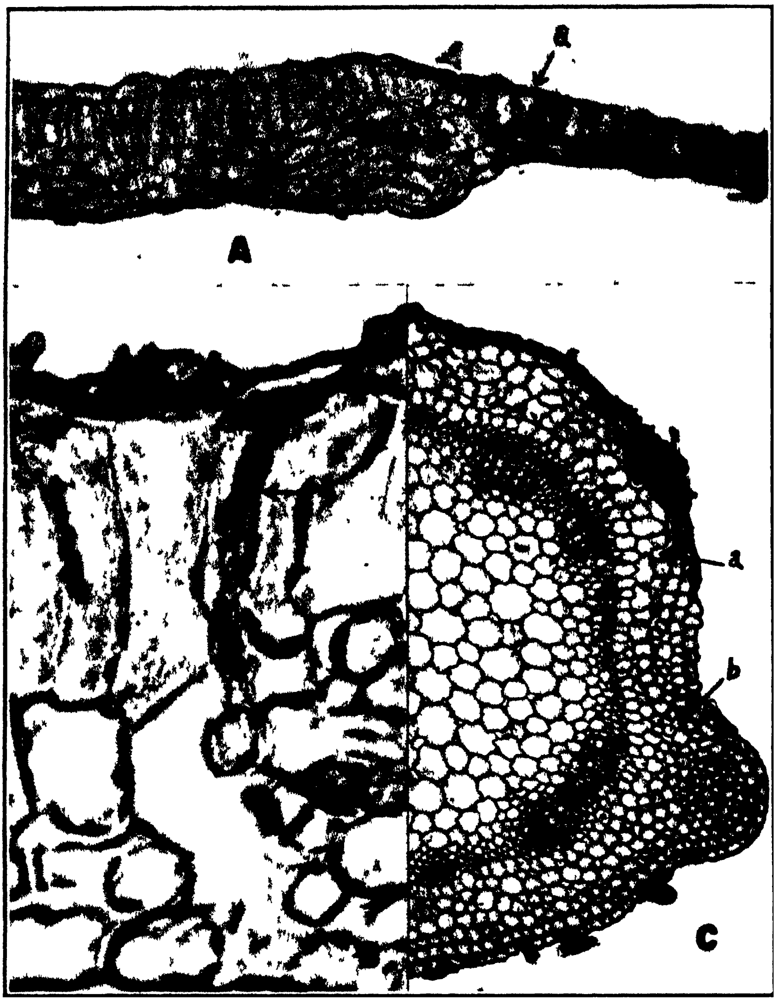

FIG. 2. Cross sections of anthracnose lesions on black peppermint leaves and stem. A. Mature lesion on leaf; note the mycelium of the anthracnose fungus on the ventral surface at a. × 150. B. Incipient infection on leaf, showing hyphae penetrating between the palisade cells at a. The epidermal cells above the penetrating hyphae have been killed × 670. C. Lesion on young stem. Infected cells are dead. Cortical parenchyma cells at a beneath the lesion have enlarged and divided; compare with normal tissues at b. × 115

$2.8 \times 6\ \mu$ and $7 \times 14\ \mu$. Recently Jenkins[4] described the fungus causing the mint anthracnose disease as *Sphaceloma menthae* Jenkins. She described the fructification as occurring in erumpent, superficial acervuli, 15–80 μ or more in diameter and on a compact, palisade of conidiophores. The writer's observations differ from the above description, in that the conidia were found to be borne on a thin, superficial fertile stroma varying up to 17 μ in thickness (Fig. 3, A, B). The conidia were sessile upon the nonerumpent stromatic layer and no hyphae were observed that could be considered conidiophores.

[4] Jenkins, Anna E. New species of *Sphaceloma* on *Aralia* and *Mentha*. Jour. Washington Acad. Sci. 27: 412–414. 1937.

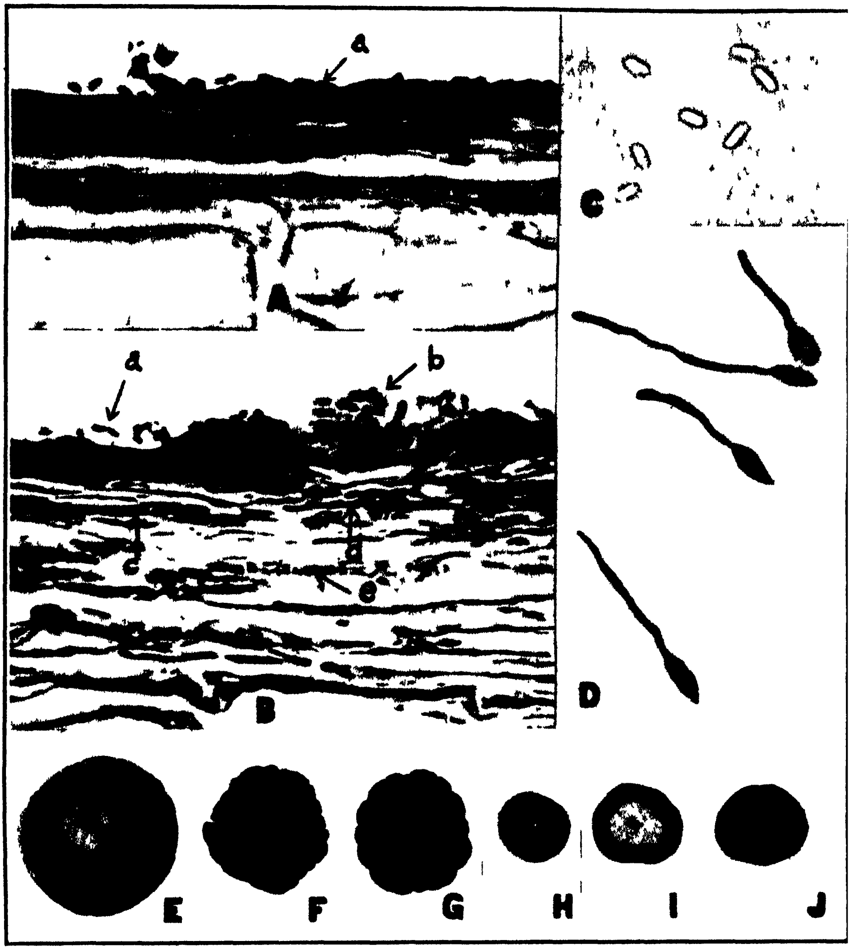

FIG. 3. A. Longitudinal section of stem lesion showing collapsed cells, and superficial stroma at a, from which conidia have been abstricted. B. Longitudinal section of stem lesion with superficial stroma and conidia at a and b, and mycelium among the collapsed cells, at c, d, and e. × 635. C. Hyaline, unicellular conidia. × 490. D. Conidia germinated in distilled water at 80° C. × 640. E–G. Three-week-old cultures on potato-2 per-cent-dextrose, 2 per cent malt-extract, and Leonian's agar media, respectively. × 1. H–J. Three week-old cultures on 2, 4, and 8 per cent oatmeal agar, respectively. The culture on 8 per cent oatmeal agar sporulated abundantly. × ½.

PATHOGENICITY ON SPECIES OF MINT

The pathogenicity of the cultures was determined by atomizing mint plants in the greenhouse and field with a suspension of spores taken from cultures growing on steamed cornstalks and on oatmeal agar. Plants inoculated in the greenhouse were placed in a moist chamber at 20° C. for 48 hours and then removed to a greenhouse maintained at 21° C. Typical infection resulted on the stems and leaves of black peppermint, following inoculation with suspensions of spores from a culture of the fungus isolated from black peppermint stems and a culture from leaves. Check plants, atomized with

distilled water, remained disease-free. The fungus was reisolated from both leaf and stem lesions of the inoculated plants. Plants of white peppermint and Japanese mint in the greenhouse, and state peppermint in field plots, also were infected by artificial inoculations with a culture isolated from black peppermint.

Later, black peppermint, Scotch and common spearmint plants in pots were inoculated in the greenhouse with cultures of the fungus isolated from Scotch spearmint and black peppermint. After 14 days, all plants inoculated with either culture were heavily infected. The check plants remained free from infection. The fungus was reisolated from each set of infected plants. No difference in the pathogenicity of the cultures was observed.

CULTURAL CHARACTERISTICS OF THE MINT ANTHRACNOSE FUNGUS

Old colonies of the fungus on potato-dextrose agar develop a reddish brown band near the margin and cause the medium to become a light greenish yellow. Since the fungus produces very few conidia on potato-dextrose agar, other media were tried. The fungus grew slowly on sterilized plugs of eggplant fruit, green tomato fruit, tomato stems, green corn stems, and old cornstalks in large test tubes, and formed a brown, warty type of growth that appeared to consist largely of isodiametric thick-wall cells resembling chlamydospores. The cultures on the pith of the sterilized green cornstalks produced an abundance of conidia. On Leonian's medium[5] a grey, heaped type of colony developed, with a dark border and no conidia (Fig. 3, G). Heaped, grey colonies, with a dark border and no conidia, were produced on agar media containing 2, 4, 6, and 8 per cent malt extract, respectively, (Fig. 3, F). On Bacto corn-meal agar and on 0.25, 0.5, and 1 per cent corn-meal agar, the colonies were prostrate, light brown and nonconidial. The fungus was grown also on 0.25, 0.5, 1.0, 2, 4, 6, and 8 per cent oatmeal media, which contained 1.0 per cent Bacto agar. Few conidia were formed by three-week-old colonies on the 1.0 per cent oatmeal medium, but were numerous on 6 and 8 per cent oatmeal media. A thin pinkish colony was formed on media containing 0.25 and 0.5 per cent oatmeal, while on media of higher concentrations of oatmeal the colonies were light colored and brown-marginate (Fig. 3, H to J). When an abundance of conidia was produced, the surface of the colonies became moist and brown.

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH AND SPORULATION OF THE FUNGUS

Duplicate cultures of the fungus were grown in Petri dishes, at 24° C., on both 6 per cent oatmeal agar and potato-2-per-cent-dextrose agar, which had been adjusted to pH values from pH 2 to pH 8.2. The pH adjustments were made by adding 0.1 N HCl or 0.1 N NaOH, to the media after sterilization. The quinhydrone electrode was used.

[5] Leonian's medium consisted of: Dry malt extract, 10 gm.; dextrose, 10 gm.; KNO_3, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 1 gm.; agar, 20 gm.; distilled water, 1 liter.

The fungus made no growth on the two media at pH 2, but grew well on both when adjusted to pH values between 3.6 and 8.0 (Table 1). In this

TABLE 1.—*Growth and sporulation of Sphaceloma sp. on media of different pH values at 24° C.*

pH value	Oatmeal agar[a]		Potato-dextrose agar[b]	
	Av. diam. of colonies	Sporulation[c]	Av. diam. of colonies	Sporulation
	mm.		*mm.*	
2.0	0	0	0	0
2.5	2	0	9	0
3.1	9	0	7	0
3.6	18	0		
4.1	19	1	26	0
4.6	20	2	30	0
5.0	20	4	32	0
5.5	21	4	33	0
6.0	19	3	32	0
6.5	17	2	30	0
7.0	20	3	33	0
7.5	15	1	35	0
8.0	20	2	33	0
8.2	19	2		

[a] Notes taken after 3 weeks.
[b] Notes taken after 4 weeks.
[c] Degree of sporulation indicated by Arabic numerals 0 to 4; 0 indicating no sporulation, 4 abundant sporulation and intermediate numerals intermediate degrees.

test the limit of tolerance to a low hydrogen-ion concentration was not reached. Sporulation was most abundant on 6 per cent oatmeal agar adjusted to pH 5 and 5.5. Few or no conidia were formed on potato-dextrose agar at any of the pH values used.

INFLUENCE OF TEMPERATURE ON GROWTH AND SPORULATION IN CULTURE

In determining the range of temperature favorable for the growth of the fungus, cultures were grown in duplicate on 1 per cent oatmeal agar at various temperatures between 2° and 33° C. Cultures on 6 per cent oatmeal agar adjusted to pH 5.5 also were placed at temperatures of 15°, 20°, 24°, and 28° C. Five dishes each containing one colony were grown at each temperature. The Petri dish cultures were kept in moist chambers to avoid drying. After 3 weeks, the diameter of the colonies at the different temperatures was measured. The results, as averages at each temperature, are presented in figure 4.

On both oatmeal media the fungus grew well at temperatures between 15° and 28° C., the optimum being at 24° C. At 2° C. the fungus grew very slowly, and at 33° C. no growth occurred. Temperatures of 15° and 20° C. were most favorable for sporulation on the 6 per cent oatmeal agar adjusted to pH 5.5, while little sporulation occurred on the 1 per cent oatmeal agar at any of the temperatures.

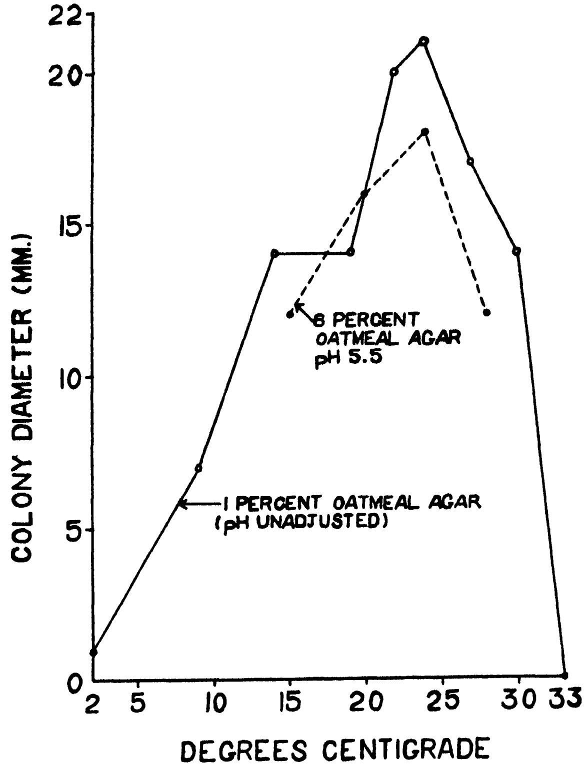

FIG. 4. Influence of temperature on the rate of growth of the mint anthracnose fungus.

OPTIMUM TEMPERATURE FOR SPORE GERMINATION

Germination tests were made in Petri dish moist chambers, each containing one glass slide on which were placed 3 separate drops of a conidial suspension from a 3-week-old culture on 6 per cent oatmeal agar. Two of the dishes, contained in a soil sampling can, were then held at each temperature of the series studied. After 20 hours, toluol was added and germination counts made. Three microscope fields, 1.43 mm. in diameter, were examined per drop, each field containing between 64 and 287 conidia. Between 2200 and 2900 spores were counted at each temperature. No spores germinated at 4.4° and 10° C. At 15.6°, 21°, 26.8°, and 32° C., 51, 88, 93, and 15 per cent, respectively, germinated. The germ tubes formed at 15.6° C. were short and sphaeroid. At 21° C. they were 3 to 6 times the length of the spores while at 26.8° C. they were somewhat shorter (Fig. 3, D), and at 32° C. were very short.

INFLUENCE OF TEMPERATURE ON INFECTION

The relation of air temperature to anthracnose infection was studied with black peppermint plants grown in 4-inch pots. Temperatures of 15.6° C. (60° F.), 21° C. (70° F.), 26.8° C. (80° F.), and 32.1° C. (90° F.) were maintained within a range of 1° C. in illuminated metal chambers. Four

plants atomized with a conidial suspension from a culture, and a check plant sprayed with water were placed at each temperature. High humidity was maintained from water in the bottom of the chambers and by atomizing the plants with distilled water 3 times a day. After 48 hours two pots of inoculated plants were removed from each temperature chamber to a greenhouse maintained at approximately 21° C., while duplicates of these were allowed to remain in the chambers for 14 days.

Seven days after inoculation, infection became evident on the plants removed from the 21° and 26.8° C. chambers after 48 hours and also on the plants held in these chambers during the test. Heavy infection subsequently developed on all the inoculated plants held in the 21° and 26.8° C. chambers for 48 hours or 14 days. Very slight infection occurred on plants held in the 32° C. chamber for 48 hours. No infection occurred on the plants maintained at that temperature for 14 days. Very slight infection developed on plants held in the 15.6° C. chamber for either 48 hours or 14 days. The check plants kept continuously in the chambers remained free from infection in all cases. A repetition of this experiment gave similar results.

OBSERVATIONS ON OVERWINTERING AND DISSEMINATION OF THE FUNGUS

Severely infected peppermint plants growing in 14-quart buckets with bottom drainage were used to study the overwintering of the fungus. On November 6, 1934, the buckets were carefully emptied, disturbing the roots and soil as little as possible. The upper portion of the soil and plants, at a depth of 13 cm. from the surface, was removed intact with a knife. The lower portion of soil and roots was returned to its original position in each bucket. Then the upper section was replaced with the tops down; the tops thus were buried 13 cm. The buckets were then sunk in the soil and remained through the winter in the open. The following April 25, the plants in 11 of the buckets were allowed to develop undisturbed. In another set of 11 buckets, the plants and plant refuse were removed and replanted with disease-free peppermint plants. In 5 buckets the soil was thoroughly stirred, as in cultivation. In 6 more buckets the soil was stirred and covered with old, infected mint refuse. The buckets were divided according to treatment and each set isolated from the other and any source of outside infection, and again sunk in soil.

On May 15, the first infection was evident, and final notes were taken on June 5. At that time abundant infection was present on the mint in the 6 buckets to which the débris had been added and on the plants in 3 of the 5 buckets in which the soil had merely been stirred. The plants in only one of the 11 buckets that were undisturbed, became infected. No infection occurred on the plants in the buckets from which diseased plants and refuse had been removed and then replanted with disease-free plants. From these results it appears that the causal organism overwinters mainly on the old mint refuse rather than in soil free from infected débris and suggests that

new shoots originating from thoroughly buried stolons only rarely become infected beneath the soil.

On June 21, 1934, a number of young mint plants were examined that had been pulled in preparation for replanting a field. The underground portions of the plants were free from any infection, while the aboveground portions of many were heavily infected. A small amount of mint débris was found on the surface of the soil, which had been insufficiently covered in the fall plowing of the field and, apparently, had served as a source of inoculum to the aerial portions. This is interpreted as additional evidence that the disease usually does not reach the surface from infected stolons underground by way of the growing shoots.

Mint débris, bearing lesions of the disease, was collected in the springs of 1935 and 1936 and examined for conidia. Conidia resembling those of the mint-anthracnose fungus were found on the surface of a few of the lesions of the previous season. The fungus was not isolated from dilution plates poured from the spores, nor was an ascogenous stage found.

Observations indicate that the disease usually is introduced into new fields by planting infected plants. Anthracnose frequently has been found uniformly scattered throughout fields of first-year peppermint. In 3 instances the disease was found on the stems and stolons of peppermint plants used for setting new fields.

DISCUSSION

The name, mint anthracnose, has been chosen for this disease in preference to "leopard spot," by which it has come to be known among some mint growers, since the preferred name has been commonly applied to diseases, of this nature, produced on other hosts by fungi of the genus *Sphaceloma*.

Mint anthracnose causes a severe loss of foliage and a cankered condition of the stems, which greatly reduce the vigor of the plants. Peppermint and spearmint oils are secreted by glands, located chiefly on the dorsal surface of the leaves. While no studies have been made of the effect of the disease on yield of oil, it appears certain that such loss of foliage and vigor must greatly reduce it. Weakened plants also may be more susceptible to winter injury. The disease has been observed to be more prevalent on peppermint than on spearmint in the commercial fields. This may be, because peppermint is the more common and more fields of it were observed. In artificial inoculations in the greenhouse, both common and Scotch spearmint were found to be as readily infected as peppermint.

The overwintering of the fungus on infected débris and the introduction of the disease into new fields on infected plants, must be considered in devising means of controlling the disease. Mint fields usually are plowed each fall to protect the plants from cold injury. This practice, if carefully performed to cover all infected mint parts at a depth of 5 to 7 inches, promises to be of value in control of the disease, since the fungus does not appear to

reach the new spring shoots from infected stems and stolons that have been thoroughly covered in the soil.

SUMMARY

Symptoms of mint anthracnose, a recently recognized disease, are described on 3 commercial varieties of peppermint, 2 strains of spearmint, and on Japanese mint. The disease causes defoliation and a severe stunting of the plants, which presumably results in reduced yield of oil.

The causal organism has been recognized as belonging to the genus *Sphaceloma*.

Similar cultures of the causal fungus were isolated from lesions on black peppermint leaves and stems and from Scotch spearmint. Typical infection resulted on the leaves and stems of 3 varieties of peppermint, Japanese mint, and 2 strains of spearmint following artificial inoculations with these cultures.

The fungus is largely intercellular and sparsely distributed in the necrotic tissue. The unicellular conidia of the fungus are sessile on a superficial stroma.

The fungus grows slowly and produces a brown, heaped type of colony on potato-dextrose, malt-extract, and Leonian's agar media, and a slow-growing, prostrate type of colony on corn-meal- and oatmeal-agar media. Conidia are produced copiously on 6 per cent oatmeal agar adjusted to pH 5.5, while few or none are produced on the other media.

The fungus grew well in culture at temperatures between 14° and 28° C., on media adjusted to pH values between 3.6 and 8.2.

Temperatures of 21° to 26.8° C. appeared to be most favorable for infection and the development of the disease.

Overwintering of the fungus was shown to occur on infected mint débris. However, when infected mint débris and living stolons are thoroughly covered with soil they do not appear to be an important source of inoculum to the new shoots in the spring.

The disease is introduced into new fields on infected plants.

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LOCAL VIRUS INFECTIONS IN RELATION TO LEAF EPIDERMAL CELLS

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INTRODUCTION

Local infections develop on leaves of certain plants after they have been wiped with extracts containing the virus of tobacco common mosaic. These infections appear as lesions several days after inoculation and their number is taken as a quantitative index of the virus (3). It has been generally considered that these local infections were caused by the virus, which enters the leaf through trichomes that are injured or broken during the wiping process of inoculation (2, 3). Sheffield (5) found that infections resulted when virus extracts were injected into trichomes by means of a micropipette and has reported (6) that there is no correlation between the number of stomata and local infections when leaves of *Nicotiana glutinosa* L. were wiped with virus extracts.

The writers have recently summarized results that indicate incidental importance of trichomes as centers for local infections when leaves are wiped with virus extracts (1). The complete, and additional, data on the relative importance of stomatal openings are here reported. The purpose of this study was to determine the relative importance of possible ways the virus may enter a leaf as it is wiped. Three possible ways of entry are considered, namely: 1, through wounds in the cells that do not project distinctly from the leaf surface and are referred to in this paper as ordinary epidermal cells; 2, through wounds in cells that project distinctly from the leaf surface and are referred to as trichomes; and 3, through stomatal openings on the leaf surface.

METHODS AND MATERIALS

Several methods were used to evaluate the trichomes as points of entry for the virus from which infection subsequently develops. First, observations were made to determine if any correlation existed between the occurrence of trichomes and evident points of local infection, after leaves had been inoculated by wiping with virus extracts. Second, inoculations were made through mutilated trichomes in one of several ways to simulate inoculations that might result when the whole leaf surface is wiped. These inoculations were so made as to avoid injuring other epidermal cells. The results when inoculations were through only broken trichomes are compared with inoculations of the whole leaf surface by wiping. Third, epidermal areas without trichomes were inoculated. The results, when the epidermis was inoculated without injury to trichomes, are compared with inoculations of the whole epidermis by wiping in the usual manner.

Inoculations to accomplish the comparisons mentioned above were made in several ways. Inoculation of the whole leaf surface was by wiping with

a swab made by tying a small wad of absorbent cotton in a piece of rayon or cheesecloth. This swab was kept saturated by frequently dipping it into the inoculum and thus thoroughly wetting the leaf surface as it was wiped. Inoculations through limited areas or cells of the leaf surface were made under a dissecting microscope. Inoculations through the ordinary cells of the leaf epidermis were made by placing a drop of virus extract on the leaf and rubbing this over the surface with a pinhead padded with rayon cloth. In this way trichomes could be avoided and the inoculations made through the other cells in a manner similar to that when the whole leaf was wiped. Another instrument was made by attaching a short piece of No. 33 constantan wire to a rigid needle. An elbow bent in this flexible wire was very useful to spread the inoculum and very lightly rub epidermal cells while avoiding trichomes. The flexible wire gave a more constant pressure and allowed more freedom of movement and vision under the dissecting microscope than when the pinhead was used.

Inoculations were made by injury to individual trichomes by 3 different methods to simulate injuries that might occur when the whole leaf surface is wiped. One method was to pinch the trichomes off the leaf with a fine-pointed forceps. This was done while the trichomes were partially immersed in the inoculum, thus insuring immediate contact of inoculum and wound. A second method was to cut the trichomes from the leaf while they were partially immersed, thus allowing the inoculum to cover the open stump of the trichome. The trichomes were cut with either of two instruments. A piece of No. 33 constantan wire was attached to a rigid needle and the end of the wire flattened and sharpened to form a small blade. This was satisfactory for cutting in cases where the trichomes were abundant and tender. When the trichomes were more sparse and tough, a larger instrument was used, made by cementing a very small chip from the edge of a safety-razor blade to a rigid wire needle. A third method of inoculating the trichomes was to break or bruise them by use of a glass rod drawn to a fine end and rounded in a flame. An elbow bent in the flexible wire instrument described above was also satisfactory for breaking and bruising the trichomes. These instruments had no sharp edges, thus they left small wounds in comparison to the larger more open wounds made by cutting as above. In all cases, when inoculating individual trichomes, care was taken not to injure other cells of the epidermis. In no case was the inoculum allowed to dry on the leaf surface before being rinsed away by water.

The inoculum was prepared by macerating and pressing the juice from leaves of mosaic-diseased tobacco plants. This was used either undiluted or in dilutions no greater than two times.

In these studies several combinations of plant species and viruses were used. Common mosaic of tobacco was used on leaves of *Nicotiana glutinosa* L., *N. rustica* L., and pepper, *Capsicum frutescens* L. A yellow-mosaic mutant of common mosaic, Type A previously described by McKinney (4) was used on leaves of *N. sylvestris* Spegaz. and Comes. Local infections were

produced by these viruses on the leaves of the respective plants. Leaves of *N. sylvestris* were used in most of the studies as a type that has numerous tender trichomes, which are readily mutilated as the leaf is lightly wiped. *N. rustica* and *N. glutinosa* are comparable to *N. sylvestris* in this respect. In contrast to the above, leaves of pepper were used as a type that has few trichomes and these are much tougher and less readily mutilated. The number of trichomes per square centimeter of leaf surface varies on the same leaf and between leaves. Sample counts to estimate an average number of trichomes per square centimeter on leaves of each of the above species show a distinct difference between the leaves of the *Nicotiana* species and the pepper. *N. sylvestris* had an average of 346 trichomes per square centimeter; *N. glutinosa,* 165; *N. rustica,* 150; and pepper, 16. The epidermis of the pepper leaf is also much tougher than that on the leaves of the *Nicotiana* species. The pepper leaves were also very satisfactory for determining the relationship between stomata and the occurrence of local lesions.

RELATION OF TRICHOMES TO LOCAL INFECTIONS

Leaves that had been inoculated by wiping with virus extract were examined to determine any relationships that might exist between the location of broken trichomes and the local lesions. The lesions were classified into 3 groups: 1, those with a trichome at or near the center; 2, those with a trichome at or near the periphery; and 3, those void of trichomes. The results of these observations are given in table 1. On the pepper leaves, which have comparatively few trichomes, 82.4 per cent of the lesions showed no relation whatever to trichomes. In comparison, on the leaves of *Nicotiana sylvestris,* which have 20 to 30 times as many trichomes per unit of area, 39.9 per cent of the lesions showed no relation to trichomes. Thus the percentage of lesions that have no relation to trichomes, decreases as the number of trichomes increases. This decrease indicates a casual rather than a causal relation between accomplishment of infections and breaking trichomes, and that infection occurs through cells of the epidermis other than trichomes when the leaves are wiped.

TABLE 1.—*Location of trichomes in relation to lesions on leaves wiped with virus inoculum*

Test plant	Relation of trichomes to lesions					
	Center		Periphery		None	
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Nicotiana sylvestris	124	39.9	63	20.2	124	39.9
Pepper	38	16.3	3	1.3	192	82.4

Tests were carried out to determine if the number of lesions is greatly reduced on leaves that have had most of their trichomes destroyed before inoculation. The right half of each *Nicotiana sylvestris* leaf was wiped

with distilled water in the manner usual for inoculation. This first wiping destroyed about 95 per cent of the trichomes, and there was very little evidence that other cells of the epidermis had been severely injured. Such leaves were allowed to stand several days before inoculation of the whole leaf by wiping with virus extract. It was found that the number of necrotic lesions was reduced only 6.8 per cent on the right halves of the leaves that were wiped twice in comparison with the left halves of the leaves, which were wiped only at the time of inoculation.

The above test was repeated, except that fine carborundum dust and distilled water were used to rub the right halves of the leaves several days before inoculation. This first wiping destroyed about 98 per cent of the trichomes, and many ordinary cells of the epidermis were severely injured or killed. After inoculation it was found that the number of necrotic lesions was reduced 31 per cent on the twice-wiped right halves in comparison with the left halves, which were wiped only at the time of inoculation (Table 2).

TABLE 2.—*Reduction in the number of local infections on leaves of Nicotiana sylvestris due to destruction of trichomes and to severe injury to other epidermal cells by wiping the right half of each leaf two to six days before inoculation by wiping the entire leaf*

Leaves tested	Lesions on the half leaves with no mutilated cells before inoculation	Lesions on the half leaves with trichomes mutilated before inoculation	Lesions on the half leaves with trichomes and other epidermal cells severely injured before inoculation[a]	Reduction in number of lesions due to mutilation of cells before inoculation
No.	*No.*	*No.*	*No.*	*Per cent*
30	1039	965[b]		7.1
30	905		568	37.2
17	1334	1247[b]		6.5
13	1008		759	24.7
3	160	160[b]		0.0
1	72	60[c]		16.7

[a] Rubbed the right half of each leaf with virus-free water and fine carborundum dust to destroy about 98 per cent of the trichomes and severely injure other epidermal cells.
[b] Wiped the right half of each leaf with virus free water to destroy about 95 per cent of the trichomes without evident severe injury to other cells of the epidermis.
[c] A wire blade was used to mutilate the trichomes without injury to other cells.

A smaller number of local infections occurred on the halves of the leaves that had been wiped or rubbed with water several days before the inoculation with virus extract. This is considered to be the result of destruction or severe injury to cells through which infection would normally occur on the uninjured leaf. The relative importance of trichomes and ordinary epidermal cells as points through which infections normally occur as the leaves are wiped with virus extract, is indicated by these data. Destruction of about 95 per cent of the trichomes several days before inoculation, reduced the number of local infections an average of 6.8 per cent. In comparison, destruction or severe injury to some of the ordinary epidermal cells reduced the number of local infections an average of 24.2 per cent.

Comparative tests were made with both young and old leaves of *Nicotiana rustica* and *N. glutinosa* in the first stages of senility. The number of trichomes per unit of area on the youngest leaves of these species is more than 3 times that on the old leaves. On these old leaves with fewer trichomes, the average number of lesions per unit area was from 1.5 to 9.6 times greater when inoculations were made by wiping the leaves with virus extracts diluted to 100X in water. Thus it is apparent that the number of lesions is not necessarily proportional to the number of trichomes per unit of area.

INOCULATIONS THROUGH TRICHOMES

Inoculations were made through individual trichomes by cutting or pinching counted numbers of them from the leaves under a dissecting microscope. The inoculations were made at random over the leaf, and observations were made at subsequent intervals under a dissecting microscope to check the location and number of infections with the points of inoculation. The data presented in table 3 show that infection developed from a small percentage of the inoculations through the mutilated trichomes.

TABLE 3.—*Percentages of infection that resulted when inoculations were made by mutilation of individual trichomes on leaves of Nicotiana species and pepper*

Test plant	Method of inoculation	Trichomes inoculated	Local infections	
		No.	*No.*	*Per cent*
N. sylvestris	Pinching	730	4	0.5
" "	Cutting	1500[a]	30	2.0
" "	"	1420[b]	33	2.3
N. rustica	"	30	1	3.3
Pepper	"	870	0	0.0

[a] Large trichomes.
[b] Small trichomes.

It will be noted that none of the 870 trichomes cut from the pepper leaves served as points of infection. In other tests some infections have resulted on pepper leaves when the trichomes were cut, but no accurate counts were made of the trichomes so inoculated. It is estimated that infection has developed from less than 0.5 per cent of the inoculations on pepper leaves by this method. No significant difference appeared between the percentage of infections that resulted from inoculation through either the very large or very small trichomes on the leaves of *Nicotiana sylvestris*. Pinching the trichomes from the leaves of *N. sylvestris* accomplished a lower percentage of infection than did cutting them. It is suspected this may be due in part to the tendency of the forceps to pinch and close the top of the trichome as it was torn from the leaf. These trichomes may be compared to those that are bent and compressed against the leaf as the surface is wiped.

Inoculations were made by mutilating all the trichomes in small areas on leaves of *Nicotiana sylvestris, N. glutinosa,* and *N. rustica* without evident injury to the ordinary cells of the epidermis. These areas of mutilated

trichomes were scattered over the right half of each of the leaves. The opposite halves of these leaves were inoculated by wiping the entire surface in the usual manner. The results of these inoculations are computed on a basis of the number of local infections per square centimeter and presented for comparison in table 4. When the points of entrance for the virus were

TABLE 4.—*Comparative number of local infections when leaves were wiped and when the trichomes only were inoculated*

Test plant	Control inoculation by wiping entire half leaves		Inoculation through mutilated trichomes in small areas			
	Av. infections per cm.²		Size of area	Areas[a] inoculated	Average infections per cm.²	
	No.	*Per cent*[b]	*Cm.²*	*No.*	*No.*	*Per cent*
Nicotiana sylvestris	6.0	100	.196	15[c]	2.12	35.3
N. glutinosa	11.47	100	.38	128[d]	2.57	22.4
N. rustica	4.59	100	.38	142[d]	0.55	12.0

[a] All the trichomes on these areas were inoculated.
[b] The control inoculation is considered 100 per cent in each instance for comparison with test inoculations.
[c] Trichomes were mutilated with wire blade.
[d] Trichomes were broken and bruised with fine glass instrument.

limited to broken or injured trichomes, the number of infections per unit of leaf area was much less than when the entire surface of the leaf was wiped with the virus extract. Infections developed at a small percentage of the mutilated trichomes and these infections represent a small percentage of the total number obtained by wiping the leaves.

It is possible that an injury sufficient to break a trichome is a too severe shock to the cell; thus the chances of its serving as a point of entry and establishment of the virus may be reduced. Degrees of injury to a living cell cannot be accurately evaluated. The trichomes of the species of *Nicotiana* studied were so tender that it was difficult to injure them with the instrument described without causing their complete collapse. On the pepper leaves, however, this was not true. As has been stated, cutting the trichomes from the pepper leaves gave a very small percentage of infection. Inoculations were made by bruising or breaking the trichomes on these leaves, as described above, with the elbow of fine flexible wire. However, no accurate data were obtained, since injury to ordinary epidermal cells could not be avoided with certainty. It, nevertheless, is pertinent that approximately 5 times more infections resulted from bruising than from cutting the trichomes. Severe injury, such as might occur to cells if too much pressure is used when leaves are inoculated by wiping, would not be so effective as a medium amount of pressure sufficient to slightly wound the cells.

Wisconsin-Havana seed tobacco plants were inoculated by mutilating the trichomes on 0.2 sq. cm. of a leaf on each plant. When the inoculated leaf was $5 \times 2\frac{1}{2}$ inches or larger, 49 per cent of 49 inoculations were positive and

symptoms appeared in an average of 16 days. In comparison, when the younger leaves, less than $5 \times 2\frac{1}{2}$ inches, were inoculated, 82 per cent of 66 inoculations were positive and symptoms appeared in an average of 10 days. The older leaves were not so susceptible to inoculation through the trichomes and required a longer period for development of symptoms. Inoculations into the stems of 24 plants were 100 per cent positive and required an average of 10 days for development of symptoms.

INOCULATION OF EPIDERMAL AREAS WITHOUT TRICHOMES

Since the trichomes are comparatively sparse in the epidermis of pepper leaves, they could be avoided while inoculating small areas between them. These areas were inoculated under a dissecting microscope by lightly rubbing the virus extract over them with a padded pinhead or with an elbow bent in a fine, flexible wire. The irregularity in size and shape of the areas between the trichomes did not allow their accurate measurement, but it is estimated that contact was made with approximately one-half of the entire leaf area when the padded pinhead was used. The flexible wire elbow allowed better vision and greater freedom of movement under the microscope, and made possible an effective contact with approximately two-thirds of the entire leaf area. In table 5 the results of such inoculations are compared with the results when comparable leaves were inoculated by wiping in the usual manner.

TABLE 5.—*Comparative numbers of local lesions on pepper leaves after the leaf surface was wiped with a cloth swab and after the epidermis was rubbed with a flexible wire elbow or padded pinhead in a manner to avoid striking the trichomes*

Method of inoculation	Leaves inoculated	Leaf area contacted	Average infections per leaf
	No.	*Per cent*	*No.*
Rubbed with flexible wire elbow	6	67[a]	15
Control—wiped	3	100	22
Rubbed with padded pinhead	5	50[a]	7
Control—wiped	2	100	20

[a] Estimated percentage of total leaf area touched as inoculations were made to avoid trichomes.

On these pepper leaves the number of lesions that developed after the areas between the trichomes were inoculated is comparable to the number obtained by wiping in the usual manner. These data show that when pepper leaves are wiped most of the inoculations occur through ordinary epidermal cells rather than trichomes.

Since cells of the epidermis other than the trichomes are inoculated when leaves are wiped with virus extract, some observations were made to determine the injury to these by the wiping. Pepper leaves were wiped with distilled water, after which the plants were placed in a chamber at 88° F. Leaves were then left in these chambers about 24 hours and collected in the morning before starch had accumulated from photosynthesis. They were immediately decolorized in hot acetic alcohol and stained in an iodine potassium iodide solution to determine the presence of starch. Examination

showed that the starch had been retained in severely injured cells. Such cells were not of great enough number to account for more than a small fraction of the local infections expected on such leaves if they had been wiped with an undiluted virus extract. Epidermal cells at the base of the trichomes frequently retained their starch. This was particularly true of the cells on the side of the trichomes where the acute angle had been formed with the epidermis as the leaves were wiped. Injuries less severe than were apparent under a magnification of 28 times are necessary to account for the largest part of the local infections that occur when pepper leaves are wiped with undiluted virus extract.

STOMATA IN RELATION TO LOCAL INFECTIONS

Leaves of the pepper used in these studies have very few trichomes on either the upper or lower epidermis. The number of stomata in the lower epidermis is 593 per sq. mm. and in the upper epidermis 26 per sq. mm., as determined by counts of sample areas of each. Forty-six leaves were wiped with fresh extracts containing the virus of tobacco common mosaic. In the case of each leaf one-half was wiped on the upper side and the other half, on the lower side.

Actual counts were made and it was found that 6.04 and 7.18 lesions per sq. cm. appeared on the halves wiped on the lower and upper sides, respectively. Thus it seems evident that stomata are not important loci for local necrotic lesions when the leaves are inoculated by wiping. These observations are in agreement with those made by Sheffield (6) on *Nicotiana glutinosa.* He states that the difference between the number of lesions when the upper and lower leaf surfaces are inoculated, "is probably due to there being fewer trichomes on the lower surface." However, it seems more probable that some factors other than the number of trichomes cause this difference in number of lesions.

DISCUSSION

From the observation of lesions on leaves of pepper and *Nicotiana sylvestris,* which had been inoculated by wiping, it is apparent that the greater the number of trichomes per unit of leaf area, the greater the chance that a lesion and trichome be coincident. The proportion of lesions that are coincident to injured trichomes is not in exact agreement with the relative numbers of trichomes per unit of leaf surface. For example, the leaves of *N. sylvestris* have approximately twenty times more trichomes per unit of area than the pepper but the proportion of lesions coincident to injured trichomes is only about twice as great. This is considered due to a factor of tenderness or vulnerability of the trichomes. As trichomes are inoculated separately or when whole leaves are wiped with virus extract, the relative degrees of injury cannot be accurately evaluated. However, cutting trichomes from pepper leaves, as compared to inoculation by bruising the trichomes, gave fewer infections. In the tests described in table 4 when attempt was made to inoculate by bruising the trichomes on *N. glutinosa* and *N. rustica* leaves, more trichomes were severely injured on the leaves of the latter and fewer lesions

were obtained. Thus tenderness and the resulting severe injury to a trichome may reduce the chance for accomplishment of infection at that point.

Small areas on the leaves of *Nicotiana sylvestris* were inoculated by mutilation of all the trichomes. The number of lesions per sq. cm. obtained on these areas was 35.3 per cent of the number obtained by wiping the opposite half leaf. This is consistent with the 39.87 per cent of the total lesions observed to have trichomes in their centers on wiped leaves of this species. These lesions are considered to be the result of inoculation through a broken trichome. In view of the above, it seems probable that 20.26 per cent of the lesions on wiped leaves observed with a broken trichome in their periphery are the result of inoculations through epidermal cells near the trichomes.

SUMMARY

When leaves were wiped with virus extracts the coincidence of broken trichomes and local infections seemed to be largely a matter of chance and the number of lesions was not necessarily proportional to the number of trichomes per unit of area.

There was a much greater reduction in the number of lesions obtained when both the trichomes and other epidermal cells were injured several days prior to inoculation by wiping the leaf than when injury previous to inoculation was limited to the trichomes.

Inoculations by mutilating the trichomes with special instruments without injury to other cells of the epidermis obtained a smaller number of lesions per sq. cm. than when inoculations were made by wiping the leaf surface.

The number of lesions per unit of area inoculated was not significantly different when either the whole surface of pepper leaves was wiped or limited areas without trichomes were rubbed.

Inoculations made by wiping the upper and lower surfaces of pepper leaves did not result in significantly different numbers of lesions. Since there are over 20 times as many stomata in the lower epidermis, it seems evident that they are not important loci for local infections.

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EXPERIMENTS WITH APHIDS AS VECTORS OF TULIP BREAKING[1]

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Investigations of tulip mosaic, commonly known as breaking, at the John Innes Horticultural Institution in England (2, 5, 6) and at the Oregon Agricultural Experiment Station (3, 4) have proved it to be a transmissible virosis. The history of the disease has been treated in detail by McKay and Warner (4), and McWhorter (7, 8) has recently shown that two viruses are concerned in breaking. In the typical broken tulip the two are present in balanced proportions. Aphid transmission has been reported by McKay and associates (3, 4) in Oregon, and by McKenny Hughes (5, 6) in England. This paper presents the Oregon data on aphid transmission.

MATERIALS AND METHODS

Transmission tests were carried out on tulips growing in Dutch beds in the field. The type of insect cage used covered 2 rows of 10 tulips each. The control series for each set of inoculated plants consisted of "sister bulbs," as explained in a previous paper (1). Several types of transmission tests were employed: (a) Aphids were placed on broken tulips for 13 to 29 days, then transferred to caged healthy tulips for the balance of the season; (b) colonies were developed on broken tulips in pots and a potted plant, with the insects on it, was set in each cage; (c) nonviruliferous aphids were introduced into cages in which both healthy and broken tulips were growing; and, in addition, a few cages enclosing healthy and broken tulips became naturally infested with *Macrosiphum* (*Illinoia*) *solanifolii* (Ashm.).[4] These several types of tests are indicated in tables 1 and 2.

The aphid colonies, except the colony of *Anuraphis tulipae* (Boyer), used in the 1928 and 1929 transfer series, were pure lines developed from single individuals and were proved nonviruliferous before the start of the experiments by previously feeding on healthy tulips. Mass cultures from colonies on broken tulips were used in the 1926–27 and 1927–28 tests.

FIELD TRIALS WITH APHIDS AS VECTORS

In a test conducted by the junior author in 1926, breaking was transmitted by aphids reported (3) as *Myzus solani* (Kalt.). Specimens of this colony are no longer available, and, unfortunately, no record remains of the

[1] Cooperative project of the Oregon Agricultural Experiment Station and the Bureau of Plant Industry, U. S. Department of Agriculture; experimental work conducted at Corvallis, Oreg.

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[4] All determinations of aphid species were made by Dr. P. W. Mason.

authority responsible for the identification. Subsequent tests have failed to confirm *M. solani* as a vector.

The results of 3 seasons' transmission tests[5] are shown in tables 1 and 2. Breaking never was detected during the season of inoculation; therefore, all records of transmission were made in the year following aphid exposure. Corresponding "sister bulb" (1) controls remained healthy during the following season in all experiments tabulated.

The data in tables 1 and 2 show *Macrosiphum (Illinoia) solanifolii* transmitted breaking in 12 of 28 tests, 60 of 419 plants exposed showed the disease, or 14.3 per cent. Similarly, *Myzus persicae* transmitted breaking in 7 of 10 tests, and 34 of 196 plants, or 17.4 per cent were infected. *M. solani, Anuraphis tulipae*, and *Rhopalosiphoninus tulipaella* failed to transmit breaking in any test; the numbers of plants exposed were respectively 117, 40, and 77. *M. circumflexus* apparently transmitted breaking in 1 of 5 tests, 3 of 95 plants exposed showing the disease, or 3.2 per cent. No evidence of mixture of this colony with a recognized vector species was detected on examination during the course of the test, and no other source of error in the test appears likely.

Manual transfer of supposedly viruliferous aphids was followed by erratic development of tulip breaking. Similar results were obtained in transmission studies of iris mosaic (1). The data for *Macrosiphum (Illinoia) solanifolii* and *Myzus persicae* in tables 1 and 2 show 11 of 27 tests by this method were successful, and 46 of 517 plants exposed or 8.9 per cent became infected. Some tests were highly successful (*e.g.*, nos. 53, 55, 57, 350); in these the aphids were introduced on diseased leaves, rather than brushed from them. Introducing the vector on a potted Rembrandt tulip produced infection in 4 of 5 tests and in 20 of 100 plants exposed. Irregularity in performance here may be due to differences in rate of aphid migration from the diseased plant. Nonviruliferous aphids placed in cages containing both broken and healthy tulips spread the disease in 2 of 4 tests and infected 6 of 71 plants exposed (8.5 per cent). In 2 tests that failed (nos. 332, 332a) the aphids were introduced late in the season (May 22). When broken and healthy tulips, caged together, became naturally infested with *M. (I.) solanifolii* (nos. 165, 109), 22 of 27 plants or 81.5 per cent became infected. Natural infestation presumably results in extensive spread of breaking only when the aphids become established on diseased plants early in the season. These colonies were detected April 30 and may have been present earlier.

Natural infestation by *Macrosiphum (Illinoia) solanifolii* of 19 cages enclosing 364 healthy tulips in the spring of 1928 induced no breaking in the progeny of these plants in 1929. This indicates that the potato aphid arrived at the bulb plot in a nonviruliferous condition. In the same season 85 healthy tulips were grown under cages together with broken tulips in the absence of aphids; none were broken in the following year. The way in which

[5] The authors are indebted to Dr. F. P. McWhorter for taking the records in the spring of 1930.

TABLE 1.—*Transmission of tulip breaking by aphids. Trials with Macrosiphum (Illinoia) solanifolii*

Trial number	Broken variety used as source of inoculum	Date of transfer[a]	Healthy variety exposed	Plants exposed and surviving in following season (Total number)	Plants exposed and surviving in following season (Per cent infected)
		1927 transfers—1928 results			
29	Farncombe Sanders Rembrandt	4/13/27	Clara Butt	19	5.2
2	Clara Butt #29	5/10/27	King Harold	18	5.5
5	" " "	"	Allard Pierson	19	0
8	" " "	"	Pride of Haarlem	18	0
11	" " "	"	William Copland	20	0
53	Golden Bronze	"	Clara Butt	18	38.8
54	Jupiter	"	"	19	10.5
55	Godet Parfait	"	"	18	50
56	Dr. Hardy	"	"	19	21
57	Edmee	"	"	19	31.5
		1928 transfers—1929 results			
165	King Harold	4/30/28[d]	King Harold	17	70
109	Farncombe Sanders Rembrandt	5/1/28[d]	Clara Butt	10	100
70	" " "	5/10/28	"	20	0
76	" " "	"	"	20	0
77	" " "	"	"	19	0
72	Bybloem Violet Gloriosum	"	"	19	0
73	Bizarre Zebra	"	"	19	0
157	Farncombe Sanders Rembrandt	5/23/28	Farncombe Sanders	17	0
248	" " "	"	Princess Elisabeth	20	0
224	Remembrance Rembrandt	"	Remembrance	20	0
		1929 transfers—1930 results			
266	Farncombe Sanders Rembrandt	4/20/29	Clara Butt	20	0
270	" " "	4/26/29[b]	"	20	20
274	" " "	4/26/29[b]	"	20	5
310	Remembrance Rembrandt	5/3/29[c]	Remembrance	16	19
347	Farncombe Sanders Rembrandt	5/6/29	Mrs. Moon	19	0
352	" " "	5/7/29	Bouton d'Or	20	0
332	Inglescombe Yellow	5/22/28[c]	Inglescombe Yellow	19	0
332a	" "	5/22/28[c]	"	17	0

[a] Manual transfer unless otherwise noted.
[b] Aphids introduced on broken tulip in a pot.
[c] Broken and healthy tulips caged together and nonviruliferous aphids added.
[d] Broken and healthy tulips caged together and naturally infested by aphids.

TABLE 2.—*Transmission of tulip breaking by aphids. Trials with Myzus persicae (Sulz.), M. circumflexus (Buckt.), M. solani, Anuraphis tulipae, and Rhopalosiphoninus tulipaella (Theob.)*

Trial number	Broken variety used as source of inoculum	Date of transfer[a]	Healthy variety exposed	Plants exposed and surviving in following season (Total number)	(Per cent infected)
		Myzus persicae			
33	Centenaire	4/13/27	Clara Butt	18	5.5
36	Rembrandt	4/27/27	"	20	10
37	"	3/20/28[b]	"	20	60
67	Farncombe Sanders Rembrandt	5/10/28	"	20	0
99	" " "	"	Pride of Haarlem	20	0
272	" " "	4/26/29[b]	Clara Butt	20	0
278	" " "	4/27/29[b]	"	20	15
350	" " "	5/15/29	Bouton d'Or	19	58
331	Inglescombe Yellow	5/31/29[c]	Inglescombe Yellow	19	15.8
		Myzus circumflexus			
68	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	19	0
269	Clara Butt	4/26/29[c]	"	18	0
273	Farncombe Sanders Rembrandt	4/26/29[b]	"	20	0
328	Princess Elisabeth	5/3/29[c]	Princess Elisabeth	18	16.7
351	Farncombe Sanders Rembrandt	5/7/29	Bouton d'Or	20	0
		Myzus solani			
69	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	20	0
182	" " "	5/11/28	William Pitt	20	0
268	" " "	4/26/29[b]	Clara Butt	20	0
275	" " "	4/26/29[b]	"	20	0
346	" " "	5/26/29	Mrs. Moon	19	0
330	Inglescombe Yellow	5/3/29[c]	Inglescombe Yellow	18	0
		Anuraphis tulipae			
71	Farncombe Sanders Rembrandt	5/10/28	Clara Butt	20	0
354	" " "	5/7/29	White Queen	20	0
		Rhopalosiphoninus tulipaella			
66	Farncombe Sanders Rembrandt	5/11/28	Clara Butt	20	0
271	" " "	4/26/29[b]	" "	20	0
349	" " "	5/26/29	Bouton d'Or	18	0
279	Clara Butt	5/3/29[c]	Clara Butt	19	0

[a] Manual transfer unless otherwise noted.
[b] Aphids introduced on broken tulips in pot.
[c] Broken and healthy tulips caged together and nonviruliferous aphids added.

caged plants became naturally infested with aphids was not determined, as the cages were apparently aphid-tight. In 1928 they were set in place over the tulips on February 26 to March 16 while the plants were small and inspection failed to reveal any aphids present at the time. One lot (no. 109), containing 10 healthy and 8 broken tulips, was caged January 30 before the plants had emerged; contamination with *M. (I.) solanifolii* occurred and 100 per cent transmission resulted. In the spring of 1929 identical cages were placed over tulips on March 14, and all were fumigated in place on April 2 and 3 with calcium cyanide—½ ounce per 1000 cubic feet for 4 hours—under a heavy canvas cover at a temperature of about 65° F. No natural infestations occurred within these fumigated cages.

There is some indication in the data of tables 1 and 2 that early infestation is more effective than late infestation in transmission of breaking. Three inoculations with *Macrosiphum (Illinoia) solanifolii* and *Myzus persicae* on April 15 were successful; 6 of 8 between April 16 and April 30; 10 of 22 between May 1 and May 15, but none of 5 after May 16 proved effective.

Four attempts to transfer iris mosaic to tulip by *Macrosiphum (Illinoia) solanifolii* failed. Similarly, attempts to transmit a virus to tulip from symptomless Bliss Triumph potato, and from Bliss Triumph affected with mild mosaic, crinkle, leaf roll, and rugose mosaic by *Myzus persicae* also failed. *M. circumflexus* failed to transmit lily mosaic to tulip in a single trial.

APHIDS ON TULIP BULBS IN STORAGE

A series of tests was conducted to determine whether the aphids that feed on tulip bulbs in storage are capable of transmitting tulip mosaic from bulb to bulb. Colonies of the aphids were allowed to feed on healthy tulip bulbs, transferred to bulbs of Rembrandt tulips for 17 to 26 days, then to healthy bulbs of Clara Butt for 17 to 38 days. The latter were fumigated and grown in the field for 2 seasons following these tests. All remained healthy. Forty-nine healthy tulips survived in the field after such experimental feeding of *Rhopalosiphoninus tulipaella*, 47 after such trials with *Anuraphis tulipae*, 20 after exposure to *Myzus* sp., and 20 after such a trial with an undetermined species. *Myzus persicae* found feeding on tulip bulbs in the laboratory in October was maintained on bulbs until February 15, but tests of virus transfer were not completed.

APHIDS OCCURRING ON TULIPS

Forty collections of aphids from tulip leaves identified by P. W. Mason include *Macrosiphum (Illinoia) solanifolii* 31, *Myzus persicae* 6, and *Myzus* sp. 3. On stored tulip bulbs *Rhopalosiphoninus tulipaella* was taken twice, *M. persicae* once, and an undetermined species once. *Macrosiphum* was found on tulips in Oregon in April and May (once on June 13), in Washington in May, and in California in late February and early March. The recognized vectors of tulip breaking appear to be common on tulips in nature, while the possible vectors *M. solani* and *M. circumflexus* are not represented in this series of collections from tulips.

DISCUSSION AND CONCLUSIONS

McWhorter (7, 8) has shown that the average broken tulip results from infection with 2 viruses in balanced proportions. Tulip Virus 1 or "color-removing," when predominating in the mixture, produces an accentuated white break; Tulip Virus 2 or "color-adding," when nearly pure, produces "red break" or "self break." McKenny Hughes (5, 6) has noted white break and red break in plants infected by means of aphids. In our experiments the usual result of aphid transmission was an average break, but some segregation of inoculated clumps into average and self break occurred. It is clear that the vectors, *Myzus persicae* and *Macrosiphum* (*Illinoia*) *solanifolii,* are each able to carry both of the tulip viruses, and that they are not selective for either under the conditions of our experiments.

McKenny Hughes (5, 6) found breaking appeared in the same season the tulips were inoculated, 6 weeks or less after viruliferous *Myzus persicae* or *Macrosiphum gei* (Koch) had been allowed to feed. Such current-season effects never appeared in our work, whether inoculations were made by aphids or by mechanical methods.

The present results are in accord with those of McKenny Hughes (5, 6) in establishing *Macrosiphum* (*Illinoia*) *solanifolii* (*Macrosiphum gei* of that author) and *Myzus persicae* as the principal vectors of tulip breaking. McKenny Hughes (6) has reported *Anuraphis tulipae* a definite vector of breaking in storage. We were unable to gather any evidence from our tests that this species could act as a vector, either in storage or on growing plants. On the other hand, we have an isolated record, apparently reliable, in which *Myzus circumflexus* transmitted breaking, and a report previously published (3, 4), but resting on incomplete records, of transmission by *M. solani.* The 2 species last named are not commonly found on tulips, although they fed on them readily under the conditions of our tests.

The suggestion based on the present data that late infestation did not serve to introduce the viruses of breaking into the bulbs is more clearly shown in McKenny Hughes' (6) data. The factors affecting transmission of viruses by aphids have been studied in detail by others, notably by Watson (9), since the present work was completed.

SUMMARY

Experiments at the Oregon Agricultural Experiment Station from 1926 to 1930 show that *Myzus persicae* and *Macrosiphum* (*Illinoia*) *solanifolii* are vectors of breaking in tulips.

Both these species of aphids are vectors for both the viruses of breaking distinguished by McWhorter.

Myzus circumflexus apparently transmitted breaking in one trial. No evidence was obtained to support our earlier report of transmission by *M. solani.* No evidence appeared in our tests that the bulb-infesting species *Anuraphis tulipae* or *Rhopalosiphoninus tulipaella* can transmit breaking.

No symptoms appeared during the season of inoculation in any of our experiments.

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INACTIVATION OF TOBACCO-MOSAIC VIRUS IN CURED TOBACCO LEAVES BY DRY HEAT[1]

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Dry-thermal inactivation of tobacco-mosaic virus in air-cured White Burley tobacco, apparently not reported in the literature, was determined for a series of temperatures with infectious 1882 White Burley tobacco and White Burley tobacco cured in 1936 containing 3 strains of virus: yellow, green, and burning. The purpose of this paper is to report these data.

Tobacco-mosaic virus in cured tobacco that had been kept 52 years in a box, in a dry barn, was found (4) to have retained some virulence. Mosaic leaf material, dried before exposure to 140° C. for 0.5 hour, has been reported (1) noninfectious, while that exposed for the same period at 120° C. remained infectious. Virus in expressed plant juices or aqueous dilutions of these juices (5) is inactivated at a lower temperature, nondiluted plant juice at 75° C. to 96° C. for 40 days to 1 minute, and juice diluted 1:20 in water at 68° C. to 92° C. for 20 days to 1 minute, respectively.

The relative stability of this virus in cured tobacco accounts, in part, for the prevalence of mosaic in some fields. Unpublished data collected from field observations in Kentucky over the past 12 years by the junior writers indicate that farms on which workmen use viruliferous non-manufactured tobacco while handling young plants have, on an average, a much higher percentage of field mosaic early in the season than those on which workmen

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

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either do not use tobacco or chew or smoke manufactured tobacco. It is apparent that infectious tobacco, even though several years old, prepared into twists for chewing and smoking, is a source of mosaic if workmen handle it while weeding, pulling, or otherwise handling succulent plants. The incidence of mosaic in fields might, therefore, be appreciably reduced if workmen used tobacco that had been rendered noninfectious. Steam sterilization of twist tobacco has given satisfactory results in mosaic control, but the flavor is changed sufficiently to be objectionable to some men, and steam sterilization is not always accessible to the tobacco grower. Twist tobacco containing about 10 per cent of moisture can be sterilized by heat in an ordinary baking oven. The tobacco is changed somewhat in flavor and contains a larger amount of readily water soluble material and is, therefore, stronger, but men who have used it have not objected.

MATERIALS AND METHODS

Infectious materials used in these experiments were 1936 field-grown, barn-cured White Burley tobacco containing, respectively, yellow, green, and burning strains of tobacco-mosaic virus. A part of the tobacco, after being dried 7 days at room temperature over concentrated sulfuric acid, was powdered, portioned into glass vials plugged with cotton, and exposed to heat in an electric oven. The remaining material was made into twists, which were allowed to dry in the air of the laboratory before exposure to heat. Infectious green tobacco was exposed to some of the temperatures without previous drying.

Virus infectivity of the samples was measured by the local-lesion method with primary leaves of beans of the variety Scotia. One gram of the nontreated and treated material was thoroughly macerated in 10 mls. of 0.1 molar solution of disodium phosphate (reaction of about pH 8.5) and allowed to stand an hour at room temperature. The extract from the macerated tissues was applied by means of an extract-saturated gauze bandage to the upper surface of 10 leaves of the test plants, which were then rinsed with running tap water to remove extraneous materials. Local lesions were counted 6 days after inoculation.

Total moisture content of the samples was taken as the loss in weight after heating for 7 days at 105° C. From these figures the moisture content of the samples was computed from their loss in weight during the treatments.

RESULTS

Data on the moisture content (1 determination) and virus infectivity (average of 3 tests) of the various samples are recorded in table 1. They indicate a similarity for tolerance to dry heat among the 3 strains of virus in cured tobacco. At 70° C. all the samples remained infectious after the maximal exposure of 60 hours, although there was a reduction of infectivity with duration of exposure. At 80, 90, 100, 110, 120, 130, 140, and 150 degrees C. complete inactivation times were 50, 20, 10, 5, 1, 0.5–0.4, 0.4 and 0.4

TABLE 1.—*Moisture content and virus infectivity of cured tobacco after heating at various temperatures for different periods of time*

Exposure		Moisture content[a]				Virus infectivity[b]			
Temp. (°C.)	Time (hrs.)	Yellow mosaic	Green mosaic	Burning mosaic	1882 mosaic	Yellow mosaic	Green mosaic	Burning mosaic	1882 mosaic
70	0	10.1	13.5	9.8	7.9	120.8	143.2	193.2	2.5
"	10	5.7	9.4	6.8	5.2	58.1	31.2	41.8	1.8
"	20	5.7	9.3	6.2	5.1	22.1	7.4	20.1	2.1
"	40	5.6	8.4	5.9	3.9	3.6	2.5	6.3	1.8
"	60	4.8	8.1	5.0	3.4	3.9	3.4	6.3	1.2
80	0	10.1	13.5	9.8	7.9	125.5	113.0	147.4	10.6
"	10	5.9	7.9	5.5	5.0	14.7	21.6	14.1	8.5
"	20	5.3	8.2	6.1	4.4	32.3	6.3	13.6	1.4
"	30	4.6	7.5	5.4	3.2	4.4	1.2	3.2	0.6
"	40	4.7	8.0	4.8	3.0	0.08	0.1	0.1	0.1
"	50	4.3	7.7	4.2	3.1	0.00	0.0	0.0	0.0
90	0	10.1	13.5	9.8	7.9	106.3	126.4	182.5	14.2
"	2	6.2	8.7	7.9	6.0	37.5	14.5	71.3	9.2
"	5	6.3	8.0	6.5	5.1	28.4	28.4	4.3	7.3
"	10	5.7	7.4	4.6	4.4	2.7	1.0	2.3	1.6
"	20	5.1	7.5	4.2	3.8	0.0	0.0	0.0	0.0
100	0	10.1	13.5	9.8	7.9	131.9	98.0	185.8	19.3
"	2	5.9	8.7	5.9	5.1	13.6	14.1	16.5	6.2
"	5	5.7	6.4	4.4	5.0	8.3	4.2	7.1	2.7
"	10	5.2	6.0	4.4	4.8	0.0	0.0	0.0	0.0
110	0	10.1	13.5	9.8	7.9	115.3	123.6	148.6	14.5
"	1	7.0	9.0	6.9	5.1	40.2	40.5	16.3	15.3
"	2	5.8	8.0	5.7	5.0	1.9	3.8	11.2	10.6
"	5	5.5	6.3	5.2	4.7	0.0	0.0	0.0	0.0
120	0.0	10.1	13.5	9.8	7.9	105.7	142.4	160.8	8.9
"	0.3	9.3	10.5	7.8	6.8	55.2	73.4	62.5	2.6
"	0.5	8.1	9.8	7.1	6.0	1.5	6.8	8.6	1.6
"	0.7	7.5	9.2	6.9	5.8	1.4	0.08	2.1	0.5
"	1.0	6.8	8.7	6.1	5.0	0.0	0.00	0.0	0.0
130	0.0	10.1	13.5	9.8		123.4	112.3	145.9	
"	0.2	8.6	11.0	7.9		70.2	23.8	28.8	
"	0.3	7.7	10.3	7.8		11.2	3.1	9.2	
"	0.4	7.4	9.4	7.5		1.0	0.0	0.3	
"	0.5	7.2	9.2	6.9		0.0	0.0	0.0	
140	0.0	10.1	13.5	9.8		137.4	103.9	118.3	
"	0.1	8.1	10.4	7.4		62.0	65.1	86.6	
"	0.2	7.9	8.7	7.2		7.1	14.3	19.8	
"	0.3	7.0	8.6	6.7		0.7	1.0	0.3	
"	0.4	6.9	8.1	6.8		0.0	0.0	0.0	
150	0.0	10.1	13.5	9.8		127.4	149.1	133.7	
"	0.1	7.9	9.4	7.0		88.8	86.6	74.0	
"	0.2	7.4	8.3	6.9		9.0	19.5	23.6	
"	0.3	6.8	7.9	6.5		0.07	0.3	0.1	
"	0.4	6.3	7.7	6.3		0.00	0.0	0.0	
150[c]	0.0					118.5	139.7	124.3	
"	0.1					42.3	85.1	64.3	
"	0.2					12.1	6.6	39.2	
"	0.3					8.5	1.2	2.0	
"	0.4					0.3	0.04	0.01	
"	0.5					0.0	0.00	0.00	

[a] Per cent moisture content based upon weight at 105° C. for 7 days.
[b] Average number of lesions per leaf from 3 tests on 10 leaves each.
[c] Tobacco made into twists for exposures.

hours, respectively. Partial inactivation was proportional to the length of exposure at the various temperatures.

Virus in green tobacco (92 per cent moisture) remained infectious after the maximal exposure of 60 hours at 70° and 80° C. At 90°, 100°, and 110° C. it was completely inactivated in 2, 2, and 1 hours, respectively. The presence of considerable moisture in the green tissues, no doubt, influenced the rate of inactivation of virus in this material. Green tobacco was not exposed to the other temperatures, because the plants in the field were killed by frost.

The moisture content and virus infectivity diminished with the increase of exposure time and temperature. However, a critical point of desiccation associated with inactivation of virus is not apparent from these data.

DISCUSSION

The three strains of tobacco-mosaic virus in cured tobacco possessed similar tolerance to heat. The rate of destruction and complete inactivation was in accordance with the expected relationship—increase in temperature or in time of exposure, with the other factor constant, decreased the infectivity. Complete inactivation of virus in the samples at various temperatures is shown in figure 1. At the higher temperatures, where the time

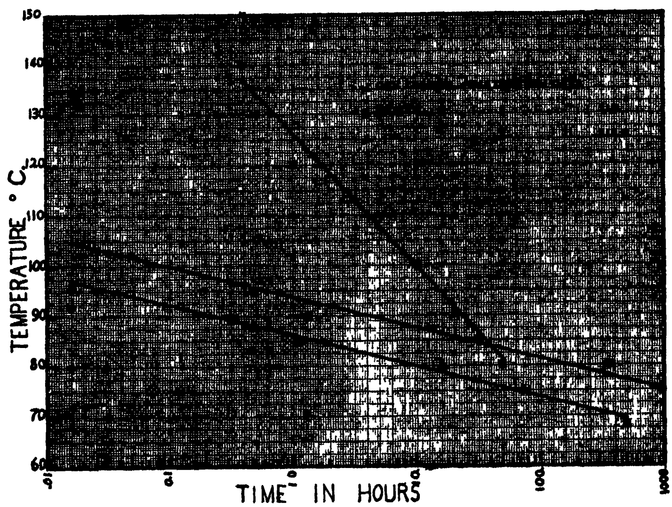

FIG. 1. Data on thermal inactivation of tobacco mosaic virus in cured tobacco and that of virus in aqueous solutions by Price (5) plotted as functions of temperature and time.

necessary for inactivation is short, the lag in inactivation is similar to that ascribed to time of penetration of heat by Bigelow (2) with thermal-death-time curves for bacteria. At the lower temperatures the time for penetration of heat has no appreciable effect because of its small proportion to the total period of exposure. Thermal inactivation of tobacco-mosaic virus in solutions (5) when plotted on the same scale (Fig. 1) shows a different rate of destruction according to the relative slope of the curves. These variations

suggest different mechanisms of inactivation the nature of which is not apparent. However, the time for inactivation of virus in solution and that in dry tissue is approximately equal at the lower temperatures. The greater effectiveness of moist heat upon inactivation is expected, since living vegetative and resting cells or native proteins are more rapidly destroyed by heat when they are in solution than when dry. Spores of *Bacillus subtilis*, previously dried (6), required 2 hours of heat at 140° C. for destruction, while proteins (egg albumen and antigens in pollen), dried and exposed to the same temperature, retained their specific antigenicity or their native configuration. According to theories, heat does not denaturize proteins when in a dry condition. Intolerance of the crystalline-protein·virus to drying at room temperature (7), destruction of virus activity by the action of certain chemicals that do not change the antigenic specificity (9), and the high molecular weight of the active protein molecule (8) indicate a unique relationship essential to the biological property of infectiousness and to regeneration within the susceptible host tissue. Since purification of the virus increases its susceptibility to inactivation upon drying, virus in expressed plant juice, or in cured or dried tissues, appears to be protected by substances or conditions present in these materials.

Inactivation of virus in the 1882-cured tobacco appears to be similar to that in tobacco recently cured. The differences in virus concentration in the nontreated tissues of the 2 tobaccos do not indicate a diminution of virus upon storage, since the virus concentration of the 1882 sample was not determined previously.

Inactivation of virus in green tissue containing 92 per cent moisture at the beginning of the experiments was, in general, intermediate between virus in solution (5) and that in dried tissues. Evaporation of water from the surface of the tissues and presence of water vapor in them probably accounted for this relationship. Since the tissues exposed at 70° and 80° C. for 60 hours lost 8.5. and 87.3 per cent of their moisture, respectively, evaporation of water from the surface may have been sufficient to prevent the heating to the point of virus inactivation, considering 80° C. for 50 hours sufficient for inactivation of virus in dried tissue. At 90°, 100°, and 110° C. the time for inactivation of virus in green tissues was less than that in dried tissues. In this case evaporation, no doubt, reduced the temperature of the tissues, but the internal areas were heated to the point of virus inactivation under the pressure of water vapor arising from water in the tissues at these temperatures. Protozoa show greater susceptibility (3) to heat destruction in moist soil (50°–60° C. for 10 minutes) than in dry soil (80°–85° C.).

Since these data indicate that tobacco-mosaic virus in cured tobacco, either in leaf or in twist form, may be inactivated (150° C. or 302° F. for 0.5 hour) within the limits of an accessible kitchen range, tobacco, to be used for chewing or smoking by workmen, may be rendered noninfectious by such a treatment at home. Since newspaper browns at this temperature, it might

be used as an indicator for the temperature to destroy virus in the absence of suitable thermometers. Importance of the destruction of virus in cured tobacco is apparent when viruliferous tobacco used by workmen appears to be the principal source of inoculum for tobacco beds and fields under conditions in Kentucky.

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HOST RANGE AND IDENTITY OF THE SMUT CAUSING ROOT GALLS IN THE GENUS BRASSICA

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INTRODUCTION

A smut causing root-galls in mustard, *Brassica campestris* L. var. *sarson* Prain, was first recorded in India by Mitra (6). Its pathogenicity was proved in pot experiments, and the smut was identified at the Imperial Mycological Institute, Kew, as *Urocystis coralloides* Rostrup. It was then supposed that the disease was confined to a single field in a village near Pusa, but affected plants have since then been collected in other villages and the disease does not seem to be so local as was once presumed. Attempts to confirm the identification and determine the host range of the smut have been made again, and the results are herewith reported.

SYMPTOMS OF THE DISEASE

In the field, affected plants are distinguishable by their stunted stature in the flowering stage. The leaves are pale and the plants apparently flower

[1] Grateful acknowledgment is made to Mr. S. F. Ashby, Director, Imperial Mycological Institute, Kew, for kindly obtaining on loan a part of Rostrup's type specimen, for going through the typescript, and for suggestions. Thanks are due to Mr. M. Azmutullah Khan of this section for the help he gave in washing the roots and in measuring the spores.

earlier than healthy ones. If pod formation has started, the pods are empty or contain very few seeds, which may be sterile. No other symptoms are evident on the parts aboveground and it is difficult to identify the disease with certainty without previous experience. If such plants are dug up by gently loosening the soil so as not to break the finer roots, large lumps of earth will be found clinging to the roots; if this soil is carefully washed out, the galls can be distinguished. If the galls are mature, they may have commenced to disintegrate; immature galls will, however, adhere to the roots. They are more numerous on the finer roots but break off easily because of their weight.

FIG. 1. A. Sarson (*Brassica campestris*) plants showing root galls. B. Sarson plants growing in non infested (pot at upper left) and smut-infested soil.

The galls are of various sizes, some as large as a pea and others measure 1½ inches in diameter. (Fig. 1, A and 2). They are irregular in outline, wart-like, cream-color when immature and lead grey, because of the presence of dark brown spores within, when mature. The surface of the galls is smooth and slightly glistening.

It is possible that mustard-crop failures attended by stunted growth of plants and reduced yields, usually ascribed to improper fertilization or other soil and environmental factors, may be due also to attack by this smut, a possibility that should not be lost sight of. An examination of the underground parts of the plants should, in such cases, invariably be made to discover if any smut galls are present.

As the disease has been seen in only a few fields, and is not easily distinguishable unless the plants are pulled out, which it is not possible to do

indiscriminately in the farmers' fields, no accurate data on losses are available. The size and number of the galls make it clear, however, that much of the food material elaborated for the production of a luxuriant growth and good yield, is used up in their formation and losses might, therefore, be appreciable.

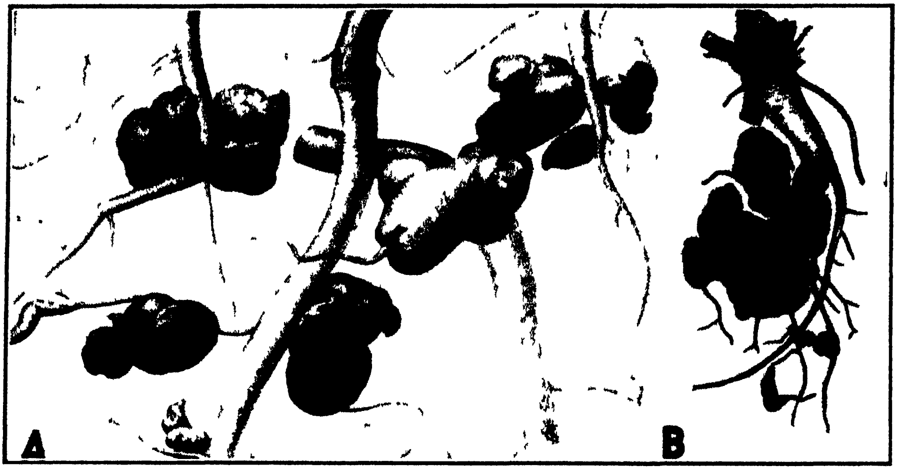

FIG. 2. A. Galls on roots, drawn from nature. ×3. B. Galls formed on *Turritis glabra* by *Urocystis coralloides*. Reproduced, with permission, from J. Lind's Danish Fungi.

POT EXPERIMENTS TO DETERMINE LOSS

In order to determine the extent of loss incurred, pot experiments were made. Soil for these experiments was obtained from a field where considerable smut was present, and a field where no such smut was found at any time. Further, in an infested field, several plants were uprooted, after harvest, and the galls collected for artificial infestation of the soil. The soil was potted as shown in table 1.

TABLE 1.—*Effect of smut infection on height and yield of mustard*

	Nature of soil	No. of pots	Total No. of plants	Mean height of plants	Average yield
				cm.	*g.*
I	Naturally infested soil	10	50	58.8	0.488
II	Same as I, but reinfested by smut	10	50	60.3	0.242
III	Noninfested soil	10	58	106.7	0.828
IV	Same as III, but artificially infested by smut	10	78	100.4	0.594

All the pots were uniformly fertilized with barnyard manure and kep moist by frequent watering throughout the summer of 1935. On Octobe 25, 5 to 8 seeds of sarson were sown in each pot. Germination was satisfac tory and the plants made normal growth until about November 10. Fron

that time onward, growth of plants in some of the pots lagged and the heights of the different plants recorded a month later showed this to be true. The results are recorded in column 4, table 1.

When the plants began to bloom, it was noted that those in infested soil flowered about 5 days earlier than those in smut-free soil. Seed formation in those plants also was indifferent. The yields per plant are recorded in the last column of table 1.

The plants were then uprooted to observe gall formation. No galls had formed in those growing in pots of series III, which contained noninfested soil, but galls were present in those growing in infested soil.

ANALYSIS OF THE RESULTS

The data recorded in table 1 indicate that the plants in naturally infested soil make poor growth, their height being especially affected. On the addition of crushed galls to soil not naturally infested, the height of the plants is lowered, presumably because of fungus infection. The data further indicate a striking decline in yield because of infection. The yield of those plants growing in naturally infested and reinfested soil was reduced by about $\frac{1}{4}$ normal, while in naturally infested soil, alone, the yield was reduced almost 50 per cent. In artificially infested disease-free soil, there was an appreciable decline.

It is further manifest from these data that galls are a factor in the dissemination of the smut. Mitra (6) was unable to induce gall formation when plants were grown in smut-free soil that had subsequently been artificially inoculated. Presumably the quantity of the inoculum used determines whether smut infection would or would not take place.

These results clearly show what a menace this smut can be to the mustard crop and point the necessity of a correct diagnosis of mustard-crop failures to determine the extent to which this smut may be responsible.

HOST RANGE OF THE SMUT

The determination of the host range of the smut among the other brassicas, many of which are useful as a source of oil seed or as vegetables, was made in 1936. The soil saved from the preceding year's pots was used for this purpose, and the following hosts were available:

Sarson (Indian mustard)	Oil seed	*Brassica campestris* L.
Black mustard	Oil seed	*Brassica nigra* Koch
Rai	Oil seed	*Brassica juncea* Coss
Toria	Oil seed	*Brassica napus* L.
Turnip	Vegetable	*Brassica rapa* var. *lorifolia* Bailey
Radish	Vegetable	*Raphanus sativus* L.
Cabbage	Vegetable	*Brassica oleracea* var. *capitata* L.

Three pots were provided for each host and in each pot about 10 plants were raised. The plants matured and were ready for examination about the end of January, when the roots were carefully washed and the galls collected. All hosts used in these tests were attacked, but the largest number

of galls was formed on sarson and turnip and the least on cabbage and toria. The galls on cabbage and toria were about 3 mm in diameter but those on sarson ranged from 8 to 22 mm.

All these hosts are therefore liable to be attacked, though the number and size of the galls formed may vary. It is further apparent that on congenial hosts the smut forms large and numerous galls, but that on less congenial hosts, gall production is very much reduced

FORMATION OF GALLS

In order to determine just when gall formation began, pot cultures of sarson plants growing in smut-infested soil were dug up every week The soil was loosened by a forcible jet of water and the rootlets were carefully collected for examination. The first galls were observed 3 weeks after sowing, in the form of irregular, flattened root swellings. They were cream white, and, on sectioning, showed the intercellular mycelium; spore formation was in its incipiency and spore balls became evident in the fourth week. Thereafter, their number gradually increased as the size of the galls increased.

The washed galls were placed for a few weeks in lacto-phenol, which served both as a killing and a fixing fluid, and then embedded in paraffin and sectioned from 5 to 10 μ thick.

In galls 3 weeks old the mycelium was confined to the cortical parenchyma where it was strictly intercellular; no haustoria were observed, but the nutritive mycelium was more prominent than the sporogenous mycelium. The host parenchyma looked as though it had been torn asunder by the invading hyphae, and large intercellular cavities full of mycelial filaments were evident here and there in the cortex. Dissolution of the middle lamella and the cell walls of the parenchyma led to an increase in size of these cavities. Sections of galls 4 weeks old showed abundant spore-ball formation. (Fig. 3). In the more advanced galls the mycelium had invaded the stelar parenchyma

The sporogenous mycelium stained a little deeper than the nutritive, and

FIG. 3. Photomicrograph of a section of a young gall. Note intercellular nutritive mycelium and spore balls.

the filaments were apparently disposed in whorls. These whorls were the spore-ball initials, and in the centre of each there were 1 to 5 prominent, thick-wall cells, the spores. The rest of the mycelium in the immediate vicinity comprised the sterile layer of cells; and all of them, the central fertile cells and the outer sterile ones, were held together firmly, forming a pseudoparenchymatous structure, the spore-ball.

As the galls increased in size and age, much of the parenchymatous tissue was replaced by the spore balls, but the stele retained its individuality, assuming a tortuously curved position within the gall. There was no abnormal increase in size of the parenchyma cells nor in their number, but there was some increase in the size of the xylem elements. The mature galls were made up of the spore balls, a hyaline fungal tissue composed of anastomosing and agglutinated mycelial strands, and the xylem tissues.

IDENTITY OF THE SMUT

In the earlier paper (6) this smut was referred to as *Urocystis coralloides*, but the appearance of the galls and the size of the spore balls suggested that it might be different. With a view to determining the validity of the earlier identification, the following points were investigated: 1. Comparison of the galls formed on *Turritis glabra* L., in Denmark and those formed in India on Indian mustard. 2. Infection experiments to determine whether the Indian smut is pathogenic to *T. glabra*. 3. Comparative study of the spores of the type material of *U. coralloides* and those of the Indian smut.

Urocystis coralloides was described by Rostrup (7) as the cause of root galls on the cruciferous plant *Turritus glabra*, in Denmark. Efforts to obtain specimens of the gall-bearing plants were unsuccessful, but, in reply to an enquiry, J. Lind, the Danish mycologist, kindly sent a copy of the illustration of the galls in his book, *Danish Fungi*. It will be noted from this (Fig. 2, B) illustration that the galls on *T. glabra* are cylindrical, unilaterally diffused enlargements, some of which are of coral-like shape. The galls formed on Indian mustard do not look like corals but are more wart-like, tuberculate, wrinkled, and globular.

Pathogenicity tests to determine whether the Indian mustard smut was or was not parasitic on *T. glabra* were rendered possible due to the kindness of the Director, Universitates Botaniska Museum of Copenhagen, Denmark, who sent seeds of that plant. Lagerheim (4) found a root-gall smut at Montpelier on *Matthiola sinuata* L., another cruciferous plant, and identified it as *Urocystis coralloides*. It is not clear from Lagerheim's account whether he had compared his smut with the type material. In these pathogenicity tests, however, plants of *M. sinuata* also were included, seeds of which were kindly supplied by the Director, Royal Botanic Gardens, Kew.

None of the seeds sown at Delhi germinated, but at Pusa 4 seeds of *Matthiola sinuata* and 7 of *Turritis glabra* germinated. The seedlings were transplanted in October, 1936, to pots containing smut-infested soil, one pot being sown to sarson, as a control. The plants were uprooted in February,

when quite mature. Galls were present in the sarson plants, but there were no galls nor even swellings seen in those of *T. glabra* and *M. sinuata*. These experiments indicated that the Indian smut was not pathogenic to either of these hosts.

A comparative study of the spores of Indian mustard smut and those of the type of *Urocystis coralloides* was rendered possible through the kindness of Mr. S. F. Ashby, Director, Imperial Mycological Institute, Kew, who obtained, on loan, a small piece of Rostrup's type specimen from the Botaniska Museum, Copenhagen. Preparations were made in lacto-phenol and measurements of 50 spore balls and over a hundred fertile and sterile spores of each specimen were made. The results are recorded in table 2.

TABLE 2.—*Linear measurements of the spore balls of the mustard smut and of Urocystis coralloides*

Materials	Indian mustard smut		*Urocystis coralloides*	
	Length	Breadth	Length	Breadth
	μ	μ	μ	μ
Sport balls				
Range	25–58	20–45	25–44	18–40
Mean	37.7	32.2	33.6	27.9
Fertile spores				
Range	13–25	10–20	14–24	10–20
Mean	20.3	15.7	19.2	15.1
Sterile spores				
Range	5–15	3–10	3–12	3–10
Mean	9.9	6.1	9.2	5.4

It will be noted from the data recorded in table 2 that the Indian smut has larger spore balls and larger fertile and sterile spores. The difference in mean values in each instance also is statistically significant.

The number of fertile spores in a spore ball has a diagnostic value in the genus, but instead of the means, the frequencies are given below, from which it will be seen that the Indian smut has a larger number of fertile spores per spore ball than has the Danish smut.

Number of cells	Indian smut; frequency	Danish smut; frequency
1	18	24
2	20	17
3	7	6
4	3	2
5	2	1

The comparative study made by the writer shows that in the shape of the galls, the nonpathogenicity of the Indian smut to the hosts to which *Urocystis coralloides* is parasitic, and in the size of the spores, the Indian smut differs from the Danish smut. It is, therefore, considered as a new species and the name *Urocystis brassicae* is proposed for it.

Urocystis brassicae n. sp. Spore powder, black, compact; spore balls 38 × 32 μ, range 25–58 × 20–45 μ with 1–5 fertile cells, 2 or 3 predominating. Fertile spores deep brown,

20 × 16 μ, range 13–25 × 9–20 μ, surrounded by numerous small, bright brown, somewhat longish, sterile spores forming a continuous layer. Spores formed in wart-like, tuberculate, irregular, lead-grey and smooth galls. Habitat: Underground parts of *Brassica campestris* L. and other *Brassica* spp.

Urocystis brassicae, species nova. Sporis atram massam pulverulentam efformantibus, presse compactis; glomerulis sporarum 38 × 32 μ, vel inter 25–58 × 20–45 μ variantibus, cellulis, fertilibus una usque ad quinque, saepius duobus vel tribus instructis. Sporis fertilibus atro-brunneis, 20 × 16 μ vel inter 13–25 × 9–20 μ variantibus, multis et parvis sterilibus sporis flavo-brunneis, longiusculis circumtectis continuum stratum efformantibus. Sporis in verruculosas et tuberculatas gallas irregulares plumbeo-glaucas leves congregatis.

Hab. in partibus subterraneis *Brassicae campestris* et aliarum specierum Brassicae.

Type specimens deposited in the Herbarium of the Imperial Agricultural Research Institute, New Delhi, Imperial Mycological Institute, Kew, and the New York Botanic Garden.

A smut, characterized by galls on the roots of yet another cruciferous plant, *Sophia andrenarum* Cockerell, was discovered in 1903 by Griffiths (3) in the United States and called by him *Urocystis sophiae*. This smut also forms wart-like tubercles, but the galls are smaller than those of the Indian smut. The spore balls of *U. sophiae* have a diameter range of 30–45 μ and the fertile cells are 15 μ in diameter. The size of the galls and that of the spores are thus much smaller than those of the Indian smut. Part of Griffiths' type specimen, secured by S. F. Ashby from G. M. Reed of the Brooklyn Botanic Garden, was lost in the mail, and no comparative study, therefore, could be made. Seed of *S. andrenarum* obtained from the United States did not germinate.

Rabenhorst founded the genus *Urocystis* to separate it from the older Friesian genus *Tuburcinia*, because the former had a continuous layer of sterile spores, apparently absent in the latter. Liro (5) contends, however, that such a layer is present in both genera and, in consequence, has merged the genus *Urocystis* into the older genus *Tuburcinia*. Germination studies by Brefeld (1, p. 180) of the spore balls of *T. primulicola* Bref., and by Woronin (8) of *T. trientalis* Berk. et Broome had shown that the peripheral cells in this genus are not sterile. Dietel (2), therefore, has retained the genus *Urocystis* and has been followed in the present paper.

SUMMARY

Symptoms of a smut that produces root galls in *Brassica campestris* are described and it is experimentally shown that considerable reduction in yields may result from attack by this fungus. The smut is soil-borne and the galls are capable of spreading the disease from place to place.

Besides Indian mustard, several other species of *Brassica*, including radish, turnip, and cabbage, are attacked by it.

The smut is not capable of infecting *Turritis glabra* nor *Matthiola sinuata*, species to which *Urocystis coralloides* is pathogenic. This fact and the spore measurements indicate that the Indian smut is not *U. coralloides* and that it is a new species. The name *Urocystis brassicae* is, therefore, proposed for it.

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THE ASSOCIATION OF BUNT WITH LOOSE SMUT AND ERGOT[1]

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(Accepted for publication Oct. 20, 1937)

The occurrence of two or more parasitic fungi on the same plant is a matter of common observation. For example, wheat plants attacked by smut may also be infected by one or more of the rusts, by mildew, and by leaf-spotting fungi. Concurrent invasion of the ovaries of plants by two parasitic fungi, however, is a phenomenon met with much less frequently. Two instances of this double infection of the ovaries of wheat observed by the writer were recorded in a brief published abstract (2). The fungi associated in the infected ovaries were those causing bunt and loose smut, and bunt and ergot. The first of these associations has been noted by other workers, but the second, so far as the writer is aware, was recorded for the first time. In the present paper it is proposed to describe briefly these double infections, and to present photographs of portions of the diseased plants.

BUNT AND LOOSE SMUT OF WHEAT

Spores of loose smut, which gain entrance to the wheat floret, germinate on the stigma about the time of anthesis and invade the young ovary. The mycelium of the parasite becomes localized in the embryo and, at the time of seed germination, passes into the growing point. Later, as the plant develops, the inflorescence is invaded and converted into a mass of spores. Not infrequently spores are produced also in the leaves and, according to Kliushnikova (3), the presence of the fungus has been established in the young roots. Infection by *Ustilago tritici* (Pers.) Rostr., therefore, may be regarded as a highly systemic invasion.

Infection by the bunt fungi, *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn, occurs soon after the wheat seed has commenced to germinate and while the young plumule is still only a few millimeters in length. As the

[1] Contribution No. 514 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa, Canada.

host plant approaches maturity its ovaries are invaded by the parasite and converted into bunt balls. The anthers of infected flowers fail to dehisce, and apparently it is the unfertilized ovary that is stimulated to develop into a bunt ball. It has occasionally been noted (1) that bunt spores are produced in the leaves as well as in the ovaries, an observation that suggests complete invasion of the infected plant. Infection of a single wheat plant by the two species of bunt may sometimes occur. Smith (6), who inoculated wheat seedlings with a mixture of *T. tritici* and *T. laevis* spores, found that approximately 50 per cent of the bunted plants were infected by both species. In those plants in which both species were present it was usually found that individual heads bore the spores of one species only, but in a few instances spores of both species were found in a single bunt ball.

Both Munerati (5) and Milan (4) have subjected loose-smut-infected seed to inoculation with bunt spores (*Tilletia tritici*). The percentage of bunt infection in plants grown from this seed was invariably less than in plants grown from seed inoculated with bunt alone. A small percentage of the seeds inoculated with the two smuts produced plants bearing both bunt and loose-smut spores in the same head.

In the summer of 1931, at Winnipeg, a few heads of Kota wheat growing in the smut plots were observed to be infected by both loose smut and bunt (*Tilletia tritici* or *T. laevis*). Usually the lower part of the spike was destroyed by loose smut, whereas the upper portion contained a few bunt balls. The seeds from which these plants grew had been artificially inoculated with spores of *T. tritici* or *T. laevis*, but the loose smut was the result of natural infection.

A few months before these observations were made, seed of Kota wheat, artificially inoculated with loose smut, was exposed to infection by bunt (*Tilletia tritici*) and grown to maturity in pots in the greenhouse. In the same experiment there were included the following lots of seed of this variety: (1) Noninoculated seed; (2) seed inoculated with *Ustilaga tritici* alone; (3) seed inoculated with *T. tritici* alone.

In the experiment just referred to, the bunt inoculations were performed by infecting young seedlings with the primary conidia (basidiospores) of *Tilletia tritici*. The lots of seed were germinated on moist sand in Petri dishes. When germination had commenced, another Petri dish containing non-nutrient agar on which bunt spores were germinating was inverted above each dish of young seedlings. This permitted the primary conidia discharged from the germinating spores to shower down upon the seedlings in the dish below. During the period of inoculation (7 days) the dishes were kept in a chamber maintained at 12°–15° C. After inoculation the seedlings were planted in the greenhouse in pots of sterilized soil. The noninoculated seed and that inoculated with *Ustilago tritici* alone were likewise germinated in moist sand and transferred to pots of sterile soil. Records were kept of the number of seeds placed to germinate, the number of plants produced, and particulars of the smut infection. The results are summarized in table 1.

TABLE 1.—*Inoculation of Kota wheat with loose smut (Ustilago tritici) and bunt (Tilletia tritici)*

Inoculation	Seeds inoculated	Plants produced	Survival	Smutted plants	Smutted plants
	No.	*No.*	*Per cent*	*No.*	*Per cent*
Noninoculated ..	100	97	97	0	0
T. tritici	112	81	72	70	86
U. tritici	100	74	74	49	66
U. tritici and *T. tritici*	100	44	44	41	93

It is a matter of some interest that the heavy smut infections, whether by *Ustilago tritici, Tilletia triticti,* or by both species, were associated with high seedling mortality. This is particularly noticeable in the lot of seedlings infected with both smuts, where only 44 per cent of the seed produced plants that survived to maturity. Results such as these show that the harmful effect of smut infection is not confined to destruction of the inflorescence, but may manifest itself also in injury to seedlings, with a consequent reduction in stand. The importance of this type of injury has been emphasized by Zade (7, 8), who suggested that the favorable effect of seed treatment on the yield of smut-resistant varieties of cereals might be due to the control of latent infection.

Table 1 shows that 41 of the 44 plants inoculated with the two species of smut became infected. The distribution of infection according to species is given in table 2. A majority of the plants showed infection with only one species of smut. The percentage infected with *Ustilago tritici* alone was about equal to that obtained from inoculation with this species only, indicating that the loose smut had not suffered from competition with bunt. The suppression of bunt in the presence of loose-smut infection was quite pronounced, as may be seen from a comparison of the precentages of plants infected with *Tilletia tritici* in tables 1 and 2. The 3 plants which produced spores of both smuts had a total of 7 heads, 4 of which were invaded by *U. tritici* and *T. tritici,* whereas the remaining 3 bore spores of *U. tritici* alone. In all of the heads infected by the two smuts the lower portion was occupied by *U. tritici* and the upper portion by *T. tritici.* The appearance of heads infected with the two smuts is shown in figure 1, A.

TABLE 2.—*Distribution of infection in plants of Kota wheat inoculated with Ustilago tritici and Tilletia tritici*

Species	Smutted plants	Smutted plants
	No.	*Per cent*
U. tritici	28	63.6
T. tritici	10	22.7
U. tritici and *T. tritici*	3	6.8
Noninfected	3	6.8

BUNT AND ERGOT

In the summers of 1931 and 1932, sclerotia of ergot, *Claviceps purpurea* (Fr.) Tul., were observed in bunted heads of wheat plants growing in the smut plots. On closer examination it was found that bunt balls containing spores of *Tilletia tritici* were fused to the projecting tips of many of the sclerotia. Composite structures of this kind were found on the following varieties of wheat: Little Club, Reward, Mindum, and Reward × Garnet (Fig. 1, B and C). The manner in which these bodies originate has not been

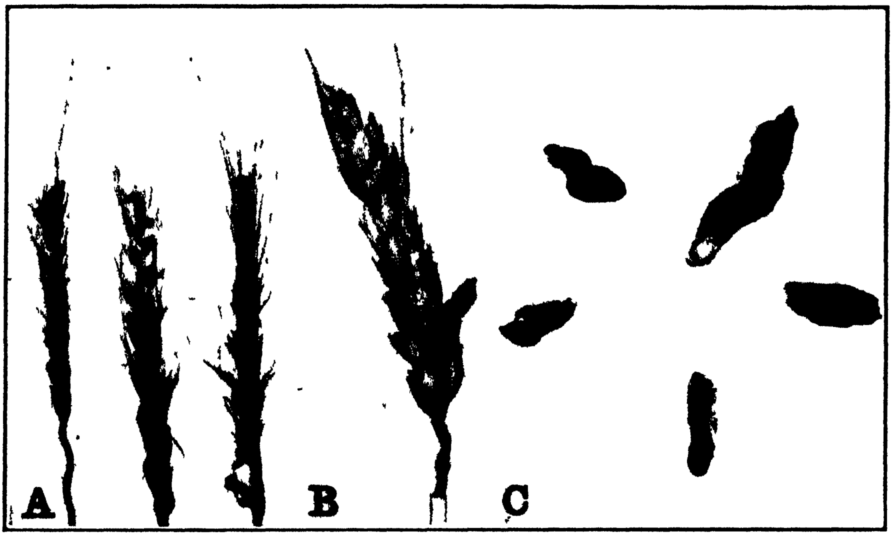

FIG. 1. A. Heads of Kota wheat infected with *Tilletia tritici* and *Ustilago tritici*. The upper portion of each head contains bunt balls. B. A bunted head of Mindum wheat. A bunt ball, fused to the tip of an ergot sclerotium, projects from one of the florets. C. Diseased wheat grains, each one composed of an ergot sclerotium and a bunt ball. The outer portion of each grain is filled with spores of *Tilletia tritici*.

investigated, but the regular occurrence of the bunt spores at the outer tip of the ergot body suggests that they may develop from partially bunted kernels. When the florets of bunted wheat plants are examined it is found that the ovaries have been invaded at a very early period in their development. It sometimes happens, however, that a part of the ovary escapes infection and, after fertilization, develops into healthy tissue. Grains of this kind in the early stages of development might be subject to infection from ascospores or conidia of the ergot fungus. This secondary infection would convert the healthy tissue into an ergot sclerotium.

SUMMARY

Heads of Kota wheat infected with both *Ustilaga tritici* and *Tilletia tritici* have been collected in the field. Heads of this variety infected by the two smuts were produced also in the greenhouse by artificial inoculation. In the material examined, bunt has been restricted to the upper and loose smut to the lower part of the spike.

The mortality is high among seedlings inoculated with both *Ustilago tritici* and *Tilletia tritici*. In greenhouse experiments only 44 per cent of the seeds inoculated with the two smuts produced mature plants, as compared with 97 per cent for noninoculated seeds. Competition between *U. tritici* and *T. tritici* resulted in a marked suppression in infection by the latter species.

On several varieties of wheat bunt balls containing spores of *Tilletia tritici* have been found fused to the projecting tips of ergot sclerotia.

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SEASONAL VARIATIONS IN SUSCEPTIBILITY OF TOBACCO TO INFECTION WITH TOBACCO-MOSAIC VIRUS

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In connection with experimental studies on the effect of mineral nutrition on host susceptibility to infection with tobacco-mosaic virus, the question arose as to whether this susceptibility is influenced also by fluctuations in environmental conditions due to seasonal changes. The object of this paper is to report briefly the results of 129 consecutive weekly tests that were carried out in an endeavor to answer this question.

MATERIALS AND METHODS

Seedlings of Turkish tobacco, *Nicotiana tabacum* L., were cultured in white quartz sand until they had developed 3 or 4 leaves and were about 2 cm. in height. Once each week 126 uniform plants at this stage of growth were transplanted into 4-inch porous clay pots, filled with composted soil, to which peat moss had been added. These pots were then placed in loose peat moss in a side bench in a greenhouse. One week the pots were placed to the left of the middle of the bench, the next week to the right, the position of the plants on the bench thus being alternated throughout the experiment.

On the 3rd day after potting, 100 plants were inoculated by means of a single puncture with a needle, size 00, in the youngest leaf over 0.5 cm. long on each plant. This method was used so that relatively uniform doses of inoculum might be introduced into each plant. The inoculations were made late in the afternoon to minimize any possible harmful effect of unfavorable light or temperature on the freshly inoculated leaf. The extract of green tobacco-mosaic virus (Johnson's tobacco virus 1) was prepared as follows: Undiluted juice from diseased tobacco plants was frozen for 48 hours in test tubes and then thawed. It was carefully remixed and 2-cc. portions placed in small test tubes. These tubes were tightly corked, sealed with paraffin, and stored in a cold room at – 14° C. until used. Each week the juice in one tube was thawed, used for the inoculation, and then discarded. In this way the virulence of virus extract was held as uniform as has been found possible. It is probable, therefore, that variations in the number of plants becoming diseased at different seasons were due to variations in host susceptibility rather than to changes in the inoculum.

The following observations and calculations were made each week: (1) incubation period of the disease, (2) number of plants infected, (3) greenhouse temperature, and (4) hours of sunshine. The temperature record was made by means of a "Tycos" recording thermometer placed on the greenhouse bench beside the test plants. The record of the hours of sunshine was obtained from a substation of the Weather Bureau of the United States Department of Agriculture, located at Trenton, N. J., 12 miles from Prince-

ton. Some variations occurred between the number of hours of sunshine in Trenton and in Princeton, but periodic checks indicate these to be quite small.

EXPERIMENTAL

The susceptibility of the young plants, measured by the percentage of plants that showed symptoms of tobacco mosaic, is expressed graphically in figure 1. Each circle on the curve represents the percentage of infection in

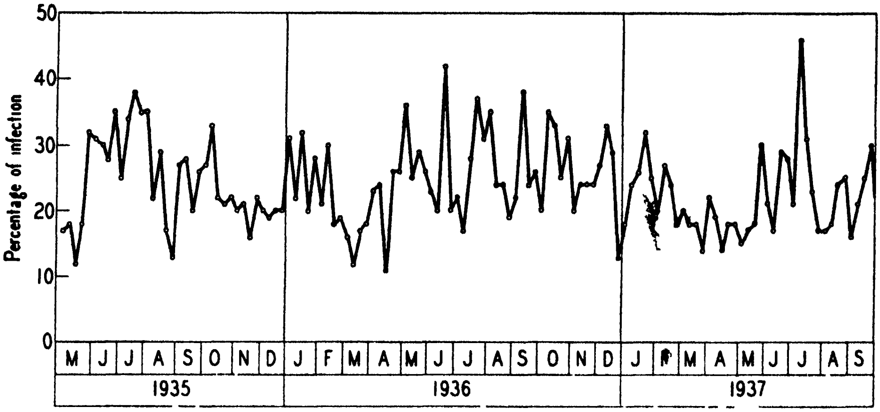

FIG. 1. Susceptibility of young tobacco plants tested at weekly intervals to infection with tobacco-mosaic virus. Each circle represents the percentage of 100 plants which became infected in each test.

a single test. From this graph it is apparent that variations in the number of plants becoming diseased were obtained from week to week. The cause of this variation is difficult to define because of the complex interrelationship of the environmental factors involved. Of these factors, the following are probably the most important: temperature, light intensity and duration, and humidity. A detailed study of these factors was not attempted because of limited equipment, but such observations as were possible with the facilities available were made on each test.

In an effort to illustrate the influence that some of these factors might exert on susceptibility, the graphs in figure 2 have been prepared. Each circle on these 4 curves represents the average of the weekly observations for each 2-month period. The upper graph shows the average hourly temperature in the greenhouse during the day of inoculation and the day following in each test. The second graph shows the average daily hours of sunshine during the same period. The third graph shows the average number of days that elapsed from time of inoculation to the appearance of symptoms of the disease. The lowermost graph shows the average susceptibility of the plants as determined by the percentage of plants that became diseased.

From the graph expressing susceptibility, it is apparent that the plants showed a somewhat definite annual cyclic variation in their susceptibility to infection with tobacco-mosaic virus. In both years the susceptibility was low during late winter and early spring, and high during early summer. In

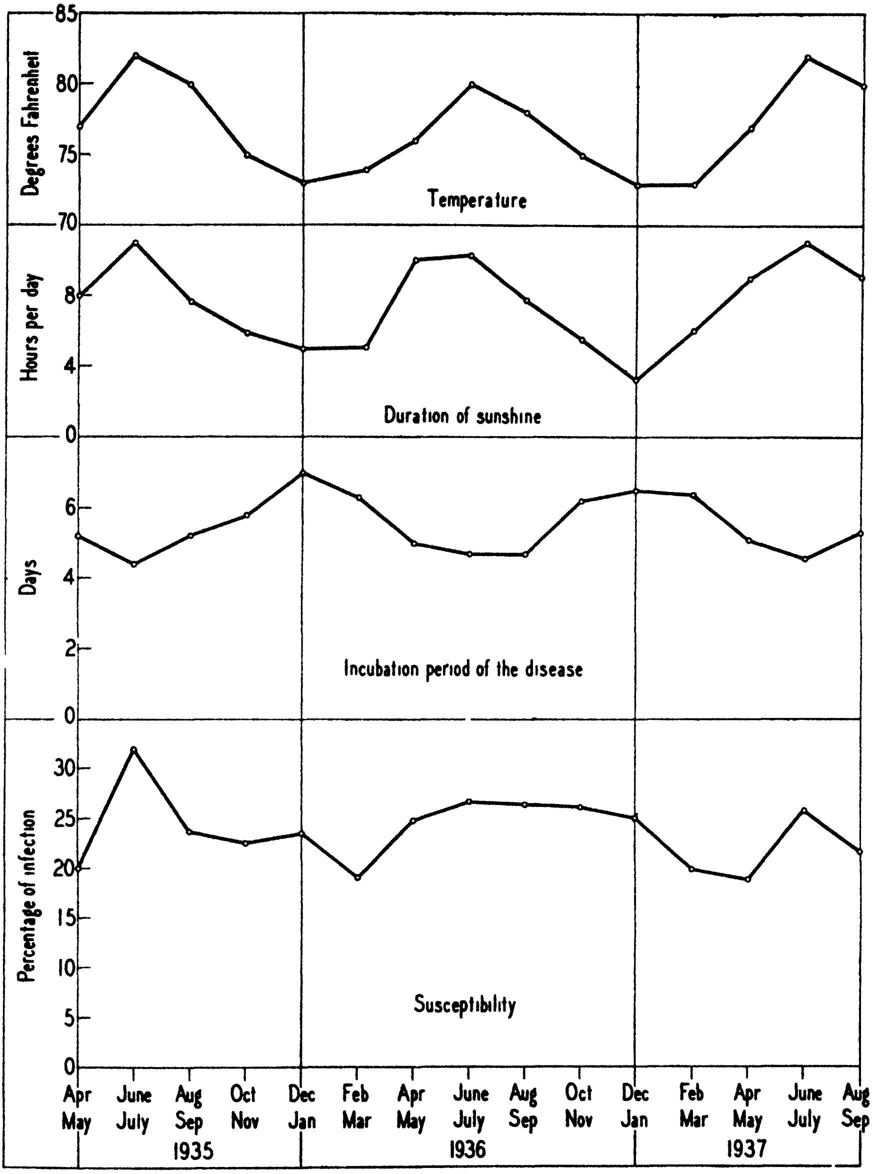

FIG. 2. Graphs showing the relation of temperature and hours of sunshine to incubation period of the disease and the susceptibility of the host. Each circle represents the average of the weekly observations made during each 2 month period.

1935, susceptibility was high during June and July and then decreased about 25 per cent during August and September The average susceptibility remained fairly constant at this level until February, 1936, when a second decrease was observed. Following an increase in susceptibility in April–May, 1936, no appreciable differences were observed until in February, 1937, when a decrease of about 20 per cent occurred. In 1937 susceptibility decreased gradually from its maximum in June and July.

In an effort to correlate to some extent these variations in susceptibility with seasonal fluctuations in temperature and light, certain salient features in the graphs in figure 2 may be mentioned. The period during which plants were low in susceptibility did not coincide with that in which the average temperature was lowest or the duration of sunshine was shortest, but followed this period by at least 2 months. The lowest average temperature and the shortest duration of sunshine occurred in the December–January period each year, but the plants were lowest in susceptibility during February–March in 1936 and during April–May in 1937. However, in both years the period of high susceptibility coincided with that of high temperature and long duration of sunshine.

The incubation period of the disease showed very marked variations from season to season, and these variations seemed constant from year to year. During June and July the symptoms of the disease appeared about 4 or 5 days after inoculation. During December and January, however, at least 6 or 7 days elapsed before the appearance of the disease. These variations seemed to be definitely correlated with seasonal fluctuations in light and temperature. The incubation period was shortest during June and July, a period characterized by high temperature and long duration of sunshine. Moreover, during December and January when the incubation period was long, the average temperature was lower and the duration of sunshine shorter than at any other time during the year.

The general results of the experiments reported here indicate that the normal variations in host reaction to tobacco-mosaic virus from season to season should be considered in planning certain types of investigations. For example, experiments in which high susceptibility to infection is desirable should be carried out during the summer months. Furthermore, the length of the incubation period of the disease is an important factor in studies pertaining to virus movement. In reporting experiments dealing with the incubation period of the disease, mention should always be made of the seasons in which such experiments were carried out.

SUMMARY

A study involving weekly tests over a period of 2½ years showed definite variations in the susceptibility of small Turkish tobacco plants to infection with tobacco-mosaic virus. Susceptibility was high during early summer, a period characterized by high temperature and long duration of sunshine, and low during late winter and early spring. The incubation period of the disease within the plant showed a direct correlation with seasonal fluctuations in light and temperature, being short during early summer and long during the winter months.

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PHYTOPATHOLOGICAL NOTES

Observations on the Loose Kernel Smut of Johnson Grass.—Although kernel smut has been observed and collected on Johnson grass, *Sorghum halepense* (L.) Pers., in the southern part of the United States for many years, there seems to be considerable confusion concerning the identity of the causal organism. In some cases it apparently has been mistaken for *Sphacelotheca sorghi* (Link) Clinton, and has been mentioned in literature under that name. Specimens of Johnson grass smut collected in Kansas in 1935 and in Oklahoma in 1936 were studied at the Kansas Agricultural Experiment Station, both in the laboratory and in the field. Samples were sent to George L. Zundel, who stated that the organism most closely resembled *S. holci* Jackson as described by H. S. Jackson[1] in 1934. The material described by Jackson was collected by Chardon, Toro, and Alamo on cultivated sorghum in Venezuela.

After the Kansas and Oklahoma specimens had been examined by Zundel and pronounced *Sphacelotheca holci*, the authors called attention to the occurrence of the fungus and stated that the report before the Kansas Academy of Science[2] was the first record of that organism on Johnson grass in North America. In a recent paper, Rodenhiser[3] calls attention to certain characteristics of a smut on Johnson grass, which he describes as a new physiologic race of the loose kernel smut, *S. cruenta* (Kühn) Potter.

Chlamydospore measurements and certain other characters of the material collected in Oklahoma and Kansas indicate that differences between this organism and *Sphacelotheca cruenta* are as wide as those between separate species of some of the other smut fungi, such as the two smuts of oats. Chlamydospores of the Johnson grass smut are larger (Fig. 1), more prominently echinulated, and somewhat darker than those of *S. cruenta*. Microscopic examination also showed that the sporidia and promycelia of the new smut are longer than those of *S. cruenta*. On the other hand, the Johnson grass smut does not differ from *S. cruenta* so sharply as certain physiologic races of *Tilletia tritici*, described by Holton,[4] differ from one another.

Among other interesting characteristics of the Johnson grass smut is its tendency to cause stunting, profuse tillering, and branching of infected plants. The dwarfing often is so severe that smutted panicles are produced on small weak tillers only a few inches tall. It also has been observed that smutted panicles appear much earlier than panicles on noninfected plants. On May 17, 1937, the senior writer observed many smutted panicles of John-

[1] Jackson, H. S. Ustilaginales. *In* Chardon, C. E., and R. A. Toro, Mycological explorations of Venezuela. Puerto Rico, Univ. Monog. Phys. and Biol. Sci. (B) no. 2. 1934.

[2] Johnston, C. O., C. L. Lefebvre, and E. D. Hansing. Kansas mycological notes, 1936. Kansas Acad. Sci. Trans. 40: [in press.]

[3] Rodenhiser, H. A. Echinulation of chlamydospores and the pathogenicity of a previously undescribed physiologic race of *Sphacelotheca cruenta*. Phytopath. 27: 643–645. 1937.

[4] Holton, C. S. Studies on seven differentiating characteristics of two physiologic forms of *Tilletia trictici*. Phytopath. 25: 1091–1098. 1935.

son grass on the roadsides between Ardmore, Oklahoma, and Dallas, Texas, but no noninfected plants were yet in head. The smutted plants were very short, the panicles usually were small, and the sori were characterized by early fragmentation of the peridium and the presence of a long prominent columella.

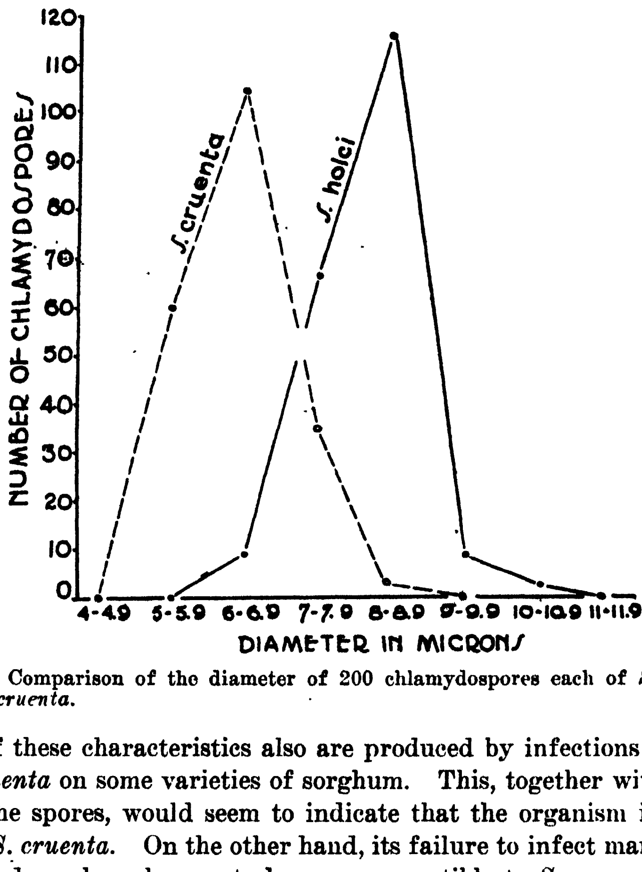

FIG. 1. Comparison of the diameter of 200 chlamydospores each of *Sphacelotheca holci* and *S. cruenta*.

Most of these characteristics also are produced by infections of *Sphacelotheca cruenta* on some varieties of sorghum. This, together with the similarity of the spores, would seem to indicate that the organism is doubtless related to *S. cruenta*. On the other hand, its failure to infect many varieties of cultivated sorghum known to be very susceptible to *S. cruenta* indicates that relationship may be rather distant. It seems, therefore, that further studies, including hybridization experiments, will be necessary to determine the identity of the organism causing loose kernel smut on Johnson grass.—C. O. JOHNSTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; C. L. LEFEBVRE, formerly Assistant Professor of Botany, and E. D. HANSING, formerly Graduate Assistant, Dept. of Botany, Kansas Agricultural Experiment Station.

Further Studies on the Parasitism of Rhizoctonia solani on Sugar Beets.[1]—In an earlier paper[2] the writer reported that none of the isolates of *Rhi-*

[1] Cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1529 of the Journal Series of the Minnesota Experiment Station.

zoctonia solani Kühn, obtained from sclerotia on potato tubers or lesions on older potato plants, caused rot of older sugar-beet roots. Subsequently, sugar-beet isolates were found to be strongly virulent, as a group, to potatoes. Therefore, it seemed probable that some potato isolates should cause rot of older sugar-beet roots. Since Rhizoctonia is frequently very destructive to potato sprouts in the spring, it was thought that the strongest likelihood of obtaining potato isolates pathogenic to sugar-beet roots would be by isolating from decaying potato sprouts.

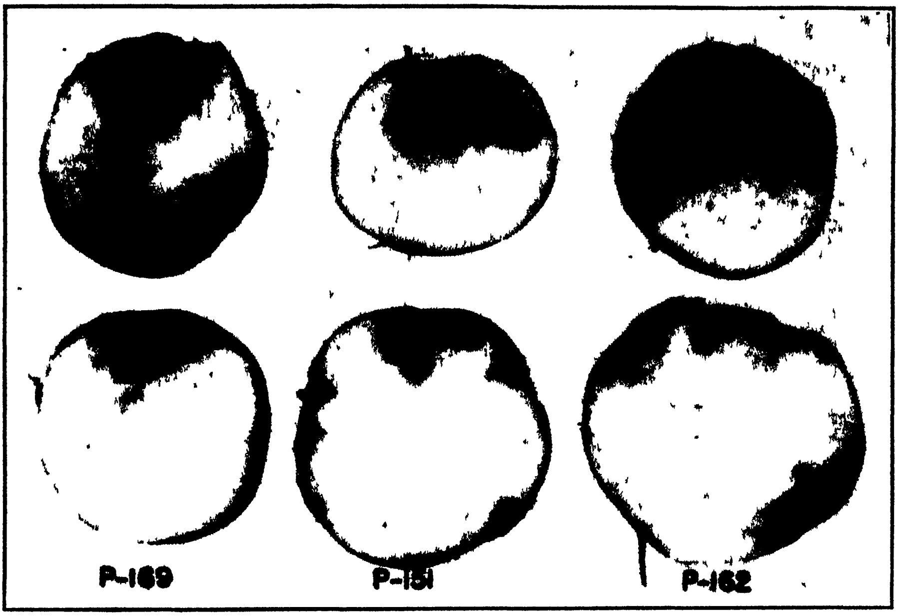

FIG. 1. Cross sections of sugar beet roots rotted by three potato sprout isolates of *Rhizoctonia solani*. Two roots rotted by each isolate are shown.

Accordingly, isolations were made from severely injured potato sprouts in the vicinity of St. Paul, Minnesota, and in Louisiana during the springs of 1935 and 1936. Three of these isolates caused appreciable decay of sugar beets (Fig. 1), and 25 additional ones caused smaller amounts of decay. However, 116 isolates from lesions on older potato plants and from sclerotia on tubers were nonpathogenic.

A more detailed account of these investigations will be published later. E. L. LECLERG, University Farm, St. Paul, Minnesota.

Crown Gall on Taxus baccata.—Crown galls have been artificially produced on different species of *Cupressus*,[1] *Libocedrus*,[2] and other conifers[3] of

[2] LeClerg, E. L. Parasitism of *Rhizoctonia solani* on sugar beet. Jour Agr. Res. [U. S.] 49: 407–431. 1934.

[1] Brown, J. G., and M. M. Evans. Crown gall on a conifer. Phytopath. 23: 97–101. 1933.

[2] Smith, C. O. Crown gall on incense cedar, *Libocedrus decurrens*. Phytopath. 27: 844–849. 1937.

[3] Smith, C. O. Crown gall on conifers. (Abstract.) Phytopath. 25: 894. 1935.

the Pinaceae, but species of the Taxaceae have not been reported as susceptible to crown gall. In table 1 are given the results of puncture inoculations

TABLE 1.—*Summary of results from inoculations on Taxus baccata var. erecta with Pseudomonas tumefaciens*

Inoculation of 1935–1936	Source of culture	Number of inoculation	Number of galls	Diameter in millimeters
January, 1935	*Prunus persica*	15	8	6–11
March	*Prunus persica*	10	7	8–15
May	*Prunus persica*	15	8	5–17
May	Control	5	0	-
June	*Prunus persica*	15	13	4–15
July	*Prunus persica*	5	5	5–13
July	Control	5	0	-
October	*Prunus persica*	5	5	8–10
August, 1936	*Libocedrus decurrens*	5	4	5–7

with *Pseudomonas tumefaciens* on European yew, *Taxus baccata* var. *erecta*. This specimen of *Taxus* was secured from a local nursery and was apparently true to type. It was a shrub 4 feet high and grown in a lathhouse. The branches inoculated were 1 year or more old. Table 1 shows that galls (Fig. 1) were developed by inoculations with cultures isolated from *Prunus*

FIG. 1. Artificial galls produced on *Taxus baccata* var. *erecta* (A, B, C, E) by cultures of *Pseudomonas tumefaciens* isolated from *Prunus persica*, and (D), galls by cultures from *Libocedrus decurrens*. A and B represent different ages of the same galls: A, after 8 months and B, after 2 years, 7 months; C, galls photographed after 2½ years; D, galls after 1 year, 3 months; E, galls after 2½ years.

persica and *Libocedrus decurrens*. About 70 per cent of the inoculations gave galls, and these galls are somewhat smaller than those usually produced on other susceptible hosts. Some were formed in nearly every series of inoculations. Under similar conditions, puncture inoculations on *Podocarpus elongata* and *Cephalotaxus fortuni* of the Taxaceae caused no galls.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

BOOK REVIEW

LEHMANN, ERNST, HANS KUMMER, and HANS DANNENMANN. *Der Schwarzrost; seine Geschichte, seine Biologie und seine Bekampfung in Verbindung mit der Berberitzenfrage.* 1st Ed. Pp. xxiv + 584. 87 text illustrations, 1 color plate. J. F. Lehmanns Verlag, München/Berlin. 1937. Price, paper covers 19.50 Rmk., cloth binding 21 Rmk.

With the spread of cereal cultivation to the far places of the earth that accompanied the extensive European migrations of last century, *Puccinia graminis* has assumed a previously unknown economic significance. Gradually a literature commensurate with its new economic importance has grown up in all the principal European languages. From time to time certain phases of the stem-rust problem have been summarized in one or another of these languages, but until the present year no attempt has been made to review thoroughly, in a work exclusively devoted to stem rust, the whole of the vast literature that has grown up around it. In recent years the necessity of such a work has been felt and suggestions have not been wanting that it should be undertaken. The appearance of *Der Schwarzrost* should therefore be hailed with satisfaction by all those connected in any way with stem rust investigations.

The work has been undertaken in no haphazard manner. The thoroughness with which the literature has been reviewed is attested by the 57-page bibliography containing references to approximately 2000 publications. The arrangement of the material gives evidence that the stem rust problem was carefully considered from its various angles before the work was undertaken. The length of the book is due largely to the anxiety of the authors that no phase of the problem should escape adequate consideration.

The book commences with a historical survey of stem-rust investigations, a survey that is to a certain extent a summary of the book as well. In this survey, as, indeed, in the book as a whole, special attention is devoted to the relationship between stem rust and the barberry. After this introductory survey the main body of the book falls into five sections; the amount of space allotted to each gives an idea of where the authors place their main emphasis: 1. The barberry (74 pages). 2. The biology of stem-rust parasitism —on both the barberry and the graminaceous hosts (228 pages). 3. Epidemiology (25 pages). 4. Distribution of stem rust and damages caused by it (40 pages). 5. The control of stem rust—with special stress placed on barberry eradication (105 pages).

The special prominence given the barberry results from the fact that recent investigations have shown that it still plays a significant part in stem-rust outbreaks in Germany. The whole question of its relationship to stem rust has, therefore, been treated with exceptional thoroughness. A detailed account is given of the distribution of the genus *Berberis*, particular emphasis being placed on the common barberry, its origin, distribution throughout the world, and the history of how it came to be associated with stem rust on cereals. In this connection it is interesting to note how the great epidemic in Europe in 1804 helped to establish in the minds of Banks, Windt, and others, the certainty of the relation of the rust on barberry to stem rust on cereals, although the actual proof was delayed for more than half a century. The consequences of de Bary's work are dealt with at length and a detailed history is given of the anti-barberry legislation enacted up to the present in different parts of the world. The extensive literature on the relation of the barberry to stem rust in North America is thoroughly reviewed and a full account is given of the barberry-eradication campaign in the United States and its consequences. In the discussion of the host-parasite relations of barberry and stem rust the views of Allen and Andrus on the mode of union of pycniospores and haploid hyphae are stated but no mention is made of Craigie's conception, although his report on the union of pycniospores and haploid hyphae in *P. helianthi* is cited in the bibliography.

Although much space is devoted to the barberry the longest and most important sections of the book deal with the relation of stem rust to its graminaceous hosts. The section dealing with physiologic specialization is, in fact, a complete manual or guide to the culturing of rust in the greenhouse and the determination of physiologic races of the wheat, oat, and rye varieties of stem rust. Every phase of the host-parasite relationship is considered: the entrance of the organism into and its development within the host, resistance and susceptibility of the host at various stages of growth, the influence of diverse environmental factors on the expression of the disease, and many others.

Perhaps the most striking feature of the book at first glance is the highly organized arrangement of the material, particularly in the parts dealing with the biology of parasitism. Here the phase of the rust on the grasses and that on barberry are considered separately. Following the practice of Fischer and Gäumann in their *Biologie der pflanzenbewohnenden parasitischen Pilze* the authors conduct the discussion under two main headings (1) the conditions necessary for the establishment of parasitic relations, and (2) the course of parasitic relations. The first of these headings is discussed separately from the point of view of both parasite and host. It thus becomes necessary to view certain factors first from the standpoint of the diploid phase of the rust, secondly from the standpoint of the grass host, thirdly from the point of view of the haploid phase of the rust and fourthly from the point of view of the barberry host. Accordingly, factors such as light, temperature, moisture, and so forth are sometimes considered from two or more of these viewpoints. Similarly, the infection types on cereals must, for the sake of the argument, be dealt with to a certain extent from the standpoint of the host and again, later, from that of the parasite. This natural and logical division of material leads inevitably to a considerable complexity of organization, and owing to the fact that these conditions are in many cases the same for both host and parasite a certain amount of repetition becomes unavoidable. Undue repetition is, however, skilfully avoided by the authors wherever possible. While this type of organization contributes much to the thoroughness of the discussion of the stem-rust problem, it also adds considerably to the length of the book.

In so comprehensive a survey occasional errors would be expected. For example, on p. 222 Newton, Johnson, and Brown are erroneously given credit for isolating a race of *P. graminis secalis* from a cross between the wheat and rye varieties of stem rust, and, on p. 178, a paper by W. L. Gordon is mistakenly cited in relation to the effect of carbon-dioxide concentration on rust development.

The book is adequately illustrated and clearly printed on paper of good quality, but the binding is scarcely strong enough for so bulky a volume.

The extreme thoroughness with which this compilation has been carried out makes it probable that it will serve as a basis for similar works in other languages. While it is regrettable that no such work is available in English, its existence in German is at least a partial compensation.—T. JOHNSON, Dominion Rust Research Laboratory, Winnipeg, Manitoba.

A RUST OF THE PIMENTO TREE IN JAMAICA, B. W. I.[1]

J. D. MACLACHLAN

(Accepted for publication October 30, 1937)

INTRODUCTION

A devastating disease of the pimento tree, *Pimenta officinalis* L., was reported from various parts of Jamaica early in 1934.[2] This disease, apparently new to science, manifested itself by blighting the foliage, young growing twigs, and inflorescences. The causal organism, *Puccinia psidii* Wint., was determined by Miss E. M. Wakefield.

This rust is of interest because it concerns certain products peculiar to Jamaica, namely, allspice, pimento oil, and a liqueur. Furthermore, the host plant belongs to the Myrtaceae, which includes several genera of economic importance. The results of the investigations now presented concern the biology of the causal organism, the factors affecting incidence of the disease, and a discussion of control methods. A preliminary report has been published.[3]

RANGE AND ECONOMIC IMPORTANCE

So far as is known, the occurrence of *Puccinia psidii* as a parasite on pimento is restricted to Jamaica. The disease caused by it made its initial appearance, in proportions to attract the attention of the grower, in April, 1934. The first outbreak occurred in the vicinity of Mandeville, and, within a remarkably short time, had spread throughout the pimento areas at the higher altitudes. Within 2 years the fruit crop in these areas was almost a complete loss, and defoliation necessitated the closing of oil distilleries. In many areas the trees are now either dead or dying from the effects of the disease.

CULTURAL TECHNIQUE

Seedling trees planted in bamboo pots were used for cultural studies in the laboratory. Prevailing high temperature made it necessary to conduct these studies under controlled temperature conditions. Consequently, a culture chamber was devised, capable of providing any desired temperature between 40° F. and 95° F. and maintaining it within 1° F. Moist absorbent

[1] The investigation was made possible by the tenure of a Sneldon Travelling Fellowship from Harvard University. For this appointment grateful acknowledgment is expressed. The writer also wishes to thank Professor J. H. Faull for many helpful suggestions and criticisms. Further appreciation is accorded for the whole-hearted support that has been given, throughout this investigation in Jamaica, by the Director of the Department of Agriculture, the Honorable A. C. Barnes, C. M. G.; the Microbiologist, Mr. F. E. V. Smith, B.Sc.; as well as the agricultural representatives and members of the planting community. All photographs, except figure 1, C, were made by Gick, photographer, Kingston, Jamaica, B. W. I.

[2] Smith, F. E. V. Rust disease of pimento. Jour. Jamaica Agr. Soc. 39: 408–411. 1935.

[3] MacLachlan, J. D. The pimento rust disease. Jour. Jamaica Agr. Soc. 40: 277–281. 1936.

cotton was wrapped around the base of the seedling. Spores were scraped from diseased leaves and either shaken up in distilled water and painted on the leaves or else dusted on leaves that had been previously atomized with distilled water. Both methods gave good results. Over the top of the seedling was inverted a small chamber lined with wet filter paper. The seedling was then placed in the culture chamber, and maintained at 60° F. for a period of 2 days, following which the cover and moist cotton were removed. The seedling was then placed in a shaded place in the garden.

Cultural studies in the field involved the following procedure: Sample branches bearing clean foliage were screened within a double layer of cheese-

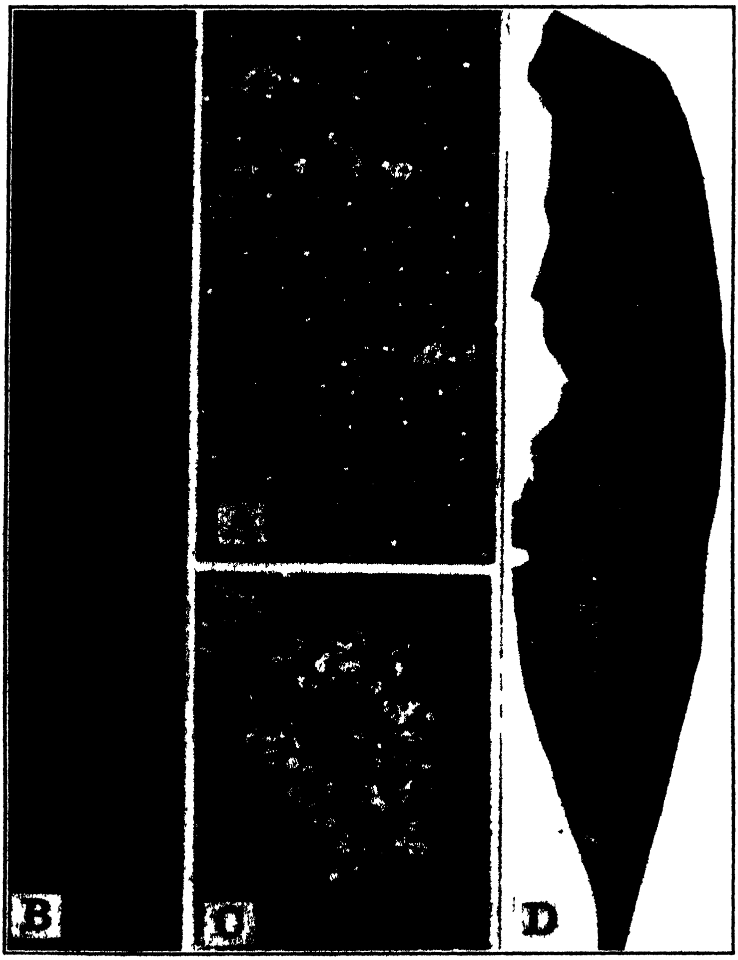

FIG. 1. Two physiological strains of *Puccinia psidii* on rose apple and pimento, respectively. A and B. Rose apple leaf that was inoculated with urediospores from a pimento leaf. This small type of lesion soon dies and never produces urediospores. Portion enlarged in A. C. A uredial lesion on pimento that resulted from inoculation with urediospores from a diseased pimento leaf. Urediospores from diseased rose apple are incapable of infecting pimento. D. Uredial lesions on rose apple leaf that was inoculated with urediospores from diseased rose apple.

cloth for a period of 5 days to eliminate interference by natural infection. Then the screen was removed and the leaves atomized with distilled water, dusted with fresh urediospores, and enclosed for 2 days within celluloid cylinders. The ends of the cylinders were plugged with moist sphagnum, and green leaves were wrapped around the cylinders to prevent direct exposure of the inoculated leaves to the sun.

BIOLOGY OF THE CAUSAL ORGANISM

Biological Specialization

There appear to be 2 distinct strains of *Puccinia psidii* parasitizing rose apple, *Eugenia jambos* L., and pimento, respectively, in Jamaica. Diseased rose apple and pimento, growing side by side, are of common occurrence. Nevertheless, urediospores from diseased rose-apple leaves were found, by inoculation, to be incapable of infecting pimento foliage. On the other hand, urediospores from diseased pimento tissues will form minute lesions (Fig. 1, A and B) on rose-apple leaves. These lesions never sporulate, and, within 2 weeks after their appearance, they turn brown and become necrotic. Similar lesions also were found in the field on rose-apple foliage growing near diseased pimento trees. Urediospores from diseased rose apple and pimento were collected at Stoney Hill, Castleton Gardens, Mavis Bank, New Castle, Content Gap, and within vicinities of Mandeville and Portland. Using the urediospores from these different sources, inoculations were made on healthy seedlings of the 2 hosts. Pimento seedlings were readily infected by urediospores from diseased pimento; the same held true for rose-apple seedlings inoculated with urediospores from diseased rose apple. In all cases, however, when cross inoculations were made, the results obtained were as indicated above.

Seedling inoculations showed that bay rum, *Pimenta acris* Kostel, was susceptible to the strain of *Puccinia psidii* parasitizing pimento, *Pimenta officinalis* (Fig. 2, B). Urediospores from the diseased bay-rum foliage were used to inoculate pimento and rose-apple seedlings. The pimento seedlings were readily infected, while the rose-apple seedlings exhibited the same type of lesion that was obtained when the inoculum from diseased pimento was used. On the other hand, the otaheite apple, *Eugenia malaccensis* L., was susceptible to the strain of *P. psidii* parasitizing rose apple, *Eugenia jambos* L. (Fig. 2, A). Urediospores from diseased otaheite-apple foliage readily infected rose apple, but were incapable of infecting pimento.

Puccinia psidii was originally described on *Psidium pomiferum* L. in Brazil by Winter.[4] *Psidium guajava* L., the common guava, was recorded as a host of this rust in Puerto Rico by Arthur.[5] According to Popenoe,[6]

[4] Winter, G. Rabenhorstii fungi europaei et extraeuropaei. Cent. XXXI. et XXXII. Hedwigia 23: 164–175. 1884.

[5] Arthur, J. C. Uredinales of Porto Rico based on collections by F. L. Stevens. [Numbers 36–75.] Mycologia 7: 227–255. 1915.

[6] Popenoe, W. Manual of tropical and subtropical fruits. . . . 474 pp. Macmillan Co., New York. 1920. (Reprinted 1924, 1927, 1934.)

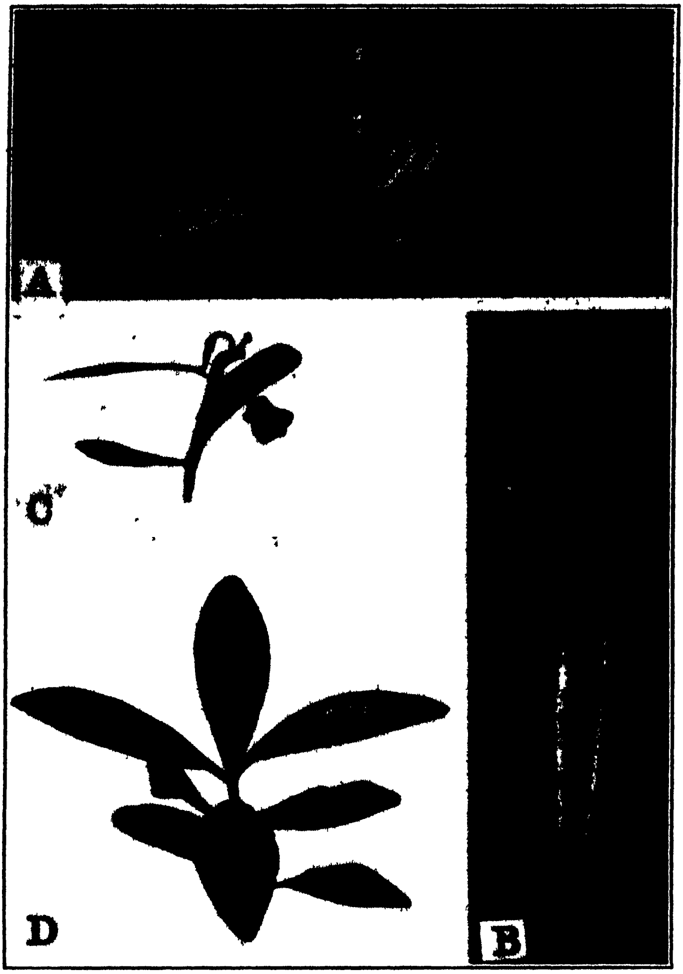

FIG. 2. A and B. Two hosts of *Puccinia psidii* hitherto unreported. A. Diseased seedling of the otaheite apple (*Eugenia malaccensis*) infected by urediospores from diseased rose apple. No infection could be obtained on the older leaves. B. Diseased leaf of the bay rum (*Pimenta acris*) infected by urediospores from diseased pimento. Note the distortion from a single lesion. C and D. Effect of temperature on the incidence of the disease caused by *P. psidii*. C. Seedling prepared as that in D but kept at an estate above 1,000 feet altitude for two weeks. One diseased leaf dropped; all new leaves were killed from severe infection. D. Seedling bearing two diseased leaves was kept at sea level for two weeks. All new leaves were healthy.

p. 275, *Psidium pomiferum* is a variety of *P. guajava*. Guava trees are of common occurrence in Jamaica within regions where *Puccinia psidii* is prevalent on both rose apple and pimento. No natural infection of guava by this rust fungus was observed. Guava seedlings proved to be immune when inoculated with urediospores from diseased pimento, bay rum, rose apple, and otaheite apple, respectively. It would appear then, that the two strains of *P. psidii* now existing in Jamaica are physiologically, at least, different from the strain from which the original description was made.

Hosts and Their Relative Susceptibility

The strain of *Puccinia psidii* parasitizing species within the genus *Pimenta* is apparently more virulent with respect to its effect on the hosts than the strain parasitizing species within the genus *Eugenia*. The hosts of the 2 strains of *P. psidii* that are found in Jamaica and their relative susceptibility may be summarized as follows:

A. Hosts of the strain of *Puccinia psidii* parasitizing species within the genus *Pimenta*.
  - *Pimenta officinalis* L. Very susceptible. Determined by inoculation and from natural infection.
  - *P. acris* Kostel. Moderately susceptible. Determined by inoculation and from one diseased seedling found at Mavis Bank.
  - *Eugenia jambos* L. Resistant; lesions never sporulate and soon die. Determined by inoculation and from natural infection.
  - *E. malaccensis* L. Resistant; lesions formed similar to those on *E. jambos*. Determined by inoculation.
  - *Psidium guajava* L. Immune. Determined by inoculation and by field observations.

B. Hosts of the strain of *P. psidii* parasitizing species within the genus *Eugenia*.
  - *Eugenia jambos*. Very susceptible. Determined by inoculation and from natural infection.
  - *E. malaccensis*. Moderately susceptible. Determined by inoculation.
  - *Pimenta officinalis*. Immune. Determined by inoculation.
  - *P. acris*. Immune. Determined by inoculation.
  - *Psidium guajava*. Immune. Determined by inoculation and by field observations.

Symptomatology

The urediospores of *Puccinia psidii* are capable of infecting the young growth only of pimento. This includes the expanding foliage, the inflorescences and the succulent young twigs. Figure 3 illustrates symptoms and signs of the rust. Diseased tissues are initially manifested by the bright yellow, powdery masses of urediospores. On the foliage these spores usually are formed on the lower surface of the blade, sometimes on the upper. Light infections appear as scattered lesions varying in size from pin-point to more than a centimeter in diameter. If the infection is severe the lesions coalesce, so that the entire leaf may be covered with a mass of urediospores. The pathogen may invade any portion of the cymose inflorescences. In many cases, infection of succulent twigs appears to be systemic, as the entire new growth of a twig may exhibit urediospores (Fig. 3, A). This phenomenon may be due to the natural growth of the twig after infection has taken place.

Light foliar infection, where only scattered lesions exist, will not cause material harm. Some distortion may occur but the leaves persist for their normal life period. After the spores have been shed the lesions turn brown to black, with scarcely any evidence of rust pustules. Severe foliar infection results in death to the leaves and defoliation within a short time. Figure 3, A and B, illustrate the effect on the foliage of various degrees of infection. Premature defoliation results in the production of new foliage that in turn becomes infected. A continuance of successive attempts to produce foliage soon utilizes the reserve energy of the tree culminating in its death. During the first season following severe infection, the effect on the foliage

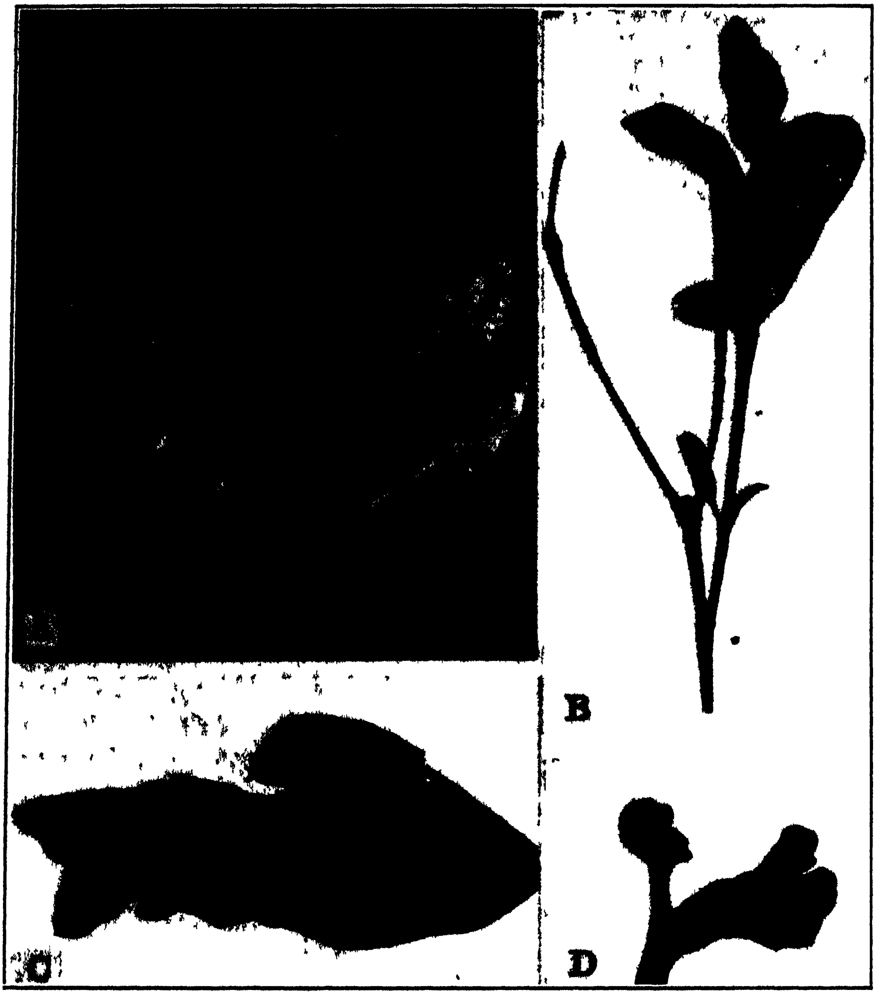

FIG. 3. Symptoms of the disease caused by *Puccinia psidii* on pimento. A. Diseased twig that illustrated various degrees of infection. The lower pair of leaves represented the type that is immune from new infections. Beyond the second pair of leaves the twig was covered with urediospores. B. Twigs that had been defoliated and killed by severe infection. C. A diseased leaf that exhibited both urediospores and teliospores. The distal half of the blade was dying. Urediospores show white in the photograph; teliospores were present at the centers of the lesions and were very abundant along the curled dead portions. D. Diseased portion of an inflorescence. Urediospores were exhibited on all portions.

is not so apparent, because the immune, one-year-old leaves still persist; these leaves, however, normally drop 2 years after their formation.

Infection of the inflorescences causes them to turn black and die. Diseased blossoms usually drop before the fruit has set. A single lesion on a pedicel is sufficient to cause that blossom to die. Infected young fruits may persist for a short time but they soon shrivel, turn black, and drop.

Infected twigs usually die because of the systemic nature of the rust within them (Fig. 3, A and B). Trees that have been subjected to severe

infection for more than a year become so weakened that twig development ceases.

Life History

Puccinia psidii apparently survives, in Jamaica, by means of the urediospore cycle only. An intensive search revealed no host for the haploid stage. The urediospore cycle involves a period of approximately two weeks. Ten to 12 days after inoculation small, watery, yellowish blisters appeared on the dorsal side of the inoculated leaves. Within 2 days these blisters erupted and exposed the bright yellow uredia. The duration of urediospore formation varies. Severe infection causes diseased leaves and blossoms to drop within 3 to 4 weeks; severely diseased twigs usually die within the same time limit; and in such cases the fungus dies with the diseased host organ. Scattered foliar lesions may cease to exhibit urediospores at their centers but continue to expand and produce them at their peripheries (Fig. 1, C) for a period of at least one month. After all the urediospores have been shed the lesions are smooth and greyish brown. They may remain in this condition for months without exhibiting any evidence of teliospore formation. Old lesions appear brownish black and are apparently necrotic.

Teliospores were very abundant on diseased pimento foliage during October and November, 1935, but were of rare occurrence during the remainder of the writer's tenure in Jamaica, namely, until April 15, 1936. Figure 3, C, illustrates a pimento leaf that is bearing urediospores on the periphery of lesions within the living portions of the leaf, and a great abundance of teliospores on old uredia within the apparently dead portions of the leaf.

FACTORS AFFECTING THE INCIDENCE OF THE DISEASE

A. EXPERIMENTAL DATA

Duration of Susceptibility of Host Tissue

Very young as well as dark green mature leaves of a pimento tree in the field were inoculated with urediospores from diseased pimento foliage. The young expanding leaves became severely diseased; the mature leaves exhibited no evidence of infection.

Leaves of various known ages on seedling pimento trees were inoculated with urediospores from diseased pimento foliage. The age of a leaf was considered to be the lapse of time between the parting of a pair of the new leaves concerned and inoculation. Data on the results obtained are given in table 1. The new leaves showed considerable variation in expansion and maturation. The majority of them ceased to expand and turned dark green 4 to 5 weeks after parting; these leaves were then immune. Others continued to expand to abnormal size and remained light yellowish green; these continued to be susceptible, but no infection was obtained on any leaves that were more than 45 days old.

TABLE 1.—*Duration of the period of susceptibility of the leaves of pimento seedlings*

Age of inoculated leaves in days[a]	Number of seedlings inoculated	Number of seedlings infected
0	8	8
20	3	3
22	3	3
24	3	3
28	3	2
32	3	3
34	3	2
35	3	2
36	3	2
37	3	0
38	3	1
40	3	1
42	3	0
45	4	1
50	3	0
53	3	0
60	7	0

[a] Leaves of seedlings that were more than 37 days old at the time of inoculation were but slightly infected. Relative to the average leaf, these leaves had grown to abnormal size and were immature when inoculated.

Relation of Time in Culture Chamber to Infection

An experiment was conducted to determine the length of time necessary for the inception of infection. The young leaves on 34 pimento seedlings were inoculated and placed in the culture chamber. At intervals of 2, 4, 6, 8, 10, 12, 14, 16, 19, 21, 24, 27, 30, 33, 36, 39 and 42 hours, respectively, two seedlings were removed from the culture chamber and exposed to a temperature of more than 75° F. where the surfaces of the inoculated leaves dried within a few minutes. Observations were made 2 weeks later. No infection was exhibited by any of the seedlings removed from the culture chamber in less than 14 hours. The seedlings incubated for 14 hours showed a few scattered lesions. A progressive increase in the number of lesions per leaf was exhibited by the seedlings incubated for 16 and 19 hours, respectively. Incubation periods of more than 19 hours resulted in no further increase in the amount of infection.

Duration of Viability of the Urediospores

Urediospores of a known age and in sufficient quantity for an experiment to determine the duration of their viability were not available. Therefore, spores were scraped in large quantity from newly formed lesions on diseased pimento foliage and mixed thoroughly. Samples of these spores were placed on clean cover glasses. Equal numbers of the prepared cover glasses were arranged in 3 separate wire trays. One tray was suspended from the roof inside a small pump-house where the daily temperature was well over 90° F. The 2 remaining trays were placed in the culture chamber at 60° F., one within a desiccator. At intervals of 2 to 3 days, 2 cover slips were removed

from each tray and the spores on each cover slip were used to inoculate individual seedlings. The viability of the spores at the time of inoculating was indicated 2 weeks later by the presence or absence of lesions on the inoculated seedlings (Table 2).

TABLE 2.—*Duration of the viability ofurediospores of P. psidii when subjected to different temperature and humidity conditions*

Time interval in days between preparation of spores and inoculation	Indication of the presence (+) or absence (0) of infection resulting from urediospores that had been kept in		
	Pump house (high temp.)	Culture chamber at 60° F.	Desiccator at 60° F.
1	+	+	Seedlings dead
2	+	+	+
4	+	+	+
5	+	+	+
7	0	+	+
9	0	+	+
11	+ very slight	+	+
14	0	0	+
16	0	+	0
19	0	0	0
22	0	+	+

Temperature Relationships

The significance of temperature and its correlative altitude on the incidence of the disease was illustrated by the following series of experiments in which susceptible young leaves of pimento seedlings were inoculated with urediospores. Six seedlings were used in each unit.

(1) Seedlings were inoculated and immediately placed in a garden at sea level where the prevailing temperatures were more than 75° F. No infection was obtained.

(2) Inoculated seedlings were incubated for two days at 60° F., then placed in the garden at sea level. Severe infection was observed on all seedlings two weeks later.

(3) Seedlings were taken to an estate of more than 1,000 feet altitude and inoculated. A maximum and minimum thermometer showed that the temperature dropped to 65° F. during the first night of incubation. Severe infection was observed 2 weeks later.

(4) The diseased seedlings of experiments (2) and (3) were used in this instance. One half of the seedlings in the garden at sea level were taken to the estate at an altitude of more than 1,000 feet. Similarly, one half of the seedlings at this estate were taken to the garden. Further data were recorded from these diseased seedlings 2 weeks later. No spread of infection was observed on the seedlings kept in the garden at sea level. The diseased leaves were almost dead but new leaves developed and remained healthy. A different picture was exhibited by the diseased seedlings left on the estate above an altitude of 1,000 feet. As new leaves

were formed at the growing point, they became heavily infected by the urediospores from diseased leaves. Figure 2, C and D, illustrates the results obtained.

An experiment was conducted to determine the range of temperatures within which infection might be obtained in the culture chamber. Seedlings were inoculated, then maintained in the culture chamber at temperatures that varied by approximately 5° F. intervals and ranged from 40° to 90° F. The experiment was performed in duplicate, and a parallel series of inoculated seedlings was maintained at 60° F. for check purposes. No infection was obtained below 47° F. Repeated and heavy infections occurred between 55° F. and 70° F. Temperatures above 70° F. inhibited infection and no infection was obtained above 80° F. All of the check seedlings exhibited heavy infection.

DISCUSSION

With Respect to the Host

The foliage is most susceptible during the period of expansion but apparently immune when the leaves have ceased to expand and have turned dark green. Whether or not the new leaves on a tree in the field will mature and become immune within the time limit exhibited by the seedlings (4 to 5 weeks) may be open to question. It is possible that under field conditions the new leaves would mature even more rapidly than those on seedlings kept under environmental conditions conducive to vigorous growth. There is some variation in the time limit of foliar maturation; field observations substantiated those made on the seedlings. This may explain, in part, the difference in the degree of infection found on the foliage of trees within the same locality. No detailed studies were made on the duration of susceptibility of inflorescences and twigs, but both are very susceptible during their earlier stages of development.

The incidence of the disease is influenced by the prevalence of susceptible host tissue. The presence of seedlings that bear large amounts of succulent growth favors the spread of the pathogen. In pimento areas that have been severely infected for some time, the trees are so weakened that there is little new growth for the pathogen to invade. At lower altitudes, where the degree of infection is less severe, the disease is most apparent in the spring when the new foliage, inflorescence, and succulent young twigs, are normally produced.

With Respect to the Pathogen

Even under optimum temperature conditions the urediospores require a period of 14 to 19 hours in a moist chamber to infect inoculated seedlings. This time limit may, in part, explain the greater prevalence of the disease found in valleys, where mists were frequently observed following a rain, and on hillsides with a northern or western exposure not directly exposed to the morning sun.

Urediospores can be found on diseased pimento foliage at any time of year but they are less abundant during the dry winter months, when little or no new growth is normally produced. The possibility of urediospores overwintering is open to question. They apparently lose their viability within a week when subjected to a temperature above 90° F. It would seem, however, that desiccation does not materially affect the viability of urediospores when subjected to a temperature of 60° F. for 3 weeks.

With Respect to Environment

Humidity. Extended periods of wet weather favor the spread of the disease. It is possible that the prevailing high humidity in 1933 favored the sudden outbreak of the rust in the spring of 1934. During 8 months (June, 1933, to March, 1934, inclusive) the rainfall varied from 50 per cent above normal to 6 times normal. The long periods of wet weather favored infection and contributed to an excess of succulent new growth. A sufficient spore population was established to cause a degree of infection that resulted in defoliation. New leaves were then formed and they, in turn, became infected. In this way a constant source of susceptible host tissue was provided for the pathogen.

Temperature. Pimento is grown from sea level to altitudes of more than 3,000 feet, and a wide range of temperatures naturally prevails. Inoculated seedlings, subjected to various temperatures in the culture chamber, indicated that the temperature range within the pimento areas is a deciding factor in the incidence of the disease. Failure to obtain infection on the inoculated seedlings below 47° F. is not significant, as such low temperature never occurs in the pimento areas. Temperature between 55° F. and 70° F. favors infection and is common at the higher altitudes. Infection is inhibited above 70° F. and such temperatures prevail at sea level.

Preliminary field surveys indicated that the disease was more prevalent at the higher altitudes. This phenomenon was substantiated by keeping inoculated seedlings (a) on an estate of more than 1,000 feet altitude and (b) at sea level. A questionnaire, requesting information concerning altitude of property, annual crop yield, and severity of the disease was sent to pimento growers. Significant replies, which gave a fair representation of the pimento areas, were received from 82. The results (Table 3) indicate that the severity of the disease is directly correlated with altitude. Below 1,000 feet, 99 per cent of the pimento was reaped where the pathogen was either absent or doing no material harm. Between 1,000 and 2,000 feet, 95 per cent of the pimento was reaped where the degree of infection was reported as mild or moderate. At altitudes of more than 2,000 feet, severe infection was reported in all cases. The severity of the disease within the 3 respective altitude limits is reflected in the crop yields of 1934 and 1935. It is also of interest to note that a relatively large percentage of the pimento is collected below an altitude of 1,000 feet, more than 40 per cent during the 4 years prior to the advent of the pathogen and more than 60 per cent afterwards.

TABLE 3.—*Data on crop yields of pimento and relative severity of the pimento rust at respective altitudes in Jamaica*

Altitude of estates in feet	Total bags collected 1930–35	Annual number of bags[a] collected						Per cent of the total number of bags collected from		Per cent of the number of bags collected in 1934 and 1935 where rust infection[b] was considered in 1936 as			
		Before advent of rust				After advent of rust							
		1930	1931	1932	1933	1934	1935	1930–33	1934–35	None	Mild	Moderate	Severe
Sea level to 1,000	13,739	2144	2366	2059	1306	4426	1438	43.3	63.6	74.6	24.2	1.2	0.0
1,000–2,000	9,984	1888	1953	1754	1678	2143	568	40.0	29.4	1.0	25.1	70.2	3.7
2,000 up	3,658	1254	675	627	462	605	35	16.7	7.0	0.0	0.0	0.0	100.0

[a] One bag contains approximately 160 pounds of the dry pimento berries.
[b] The degrees of infection were defined as:

None—No rust.
Mild—Rust is present but not doing any damage.
Moderate—Leaves infected but not killed; crop yield of one half to three quarters of the average.
Severe—Majority of trees defoliated by the rust. Fruit yield almost a total loss.

CONTROL

The feasibility of using fungicides to control the pimento rust is questionable. Most of the trees grow where it would be almost impossible to take a power sprayer. Furthermore, pimento is an extremely variable crop. Even weather conditions during blossom time seem to determine whether 200 or 20 bags will be collected. Thus, it would be difficult to convince the pimento grower to go to the expense of spraying before the fruit has set. In certain instances it might be of value to spray at intervals for 2 months during blossom time. In so doing, protection could be afforded the fruit as well as sufficient foliage to carry the tree through the year.

Although no artificial means of control was found, the pimento industry seems by no means doomed. A relatively large percentage of the pimento is grown at the lower altitudes, where the prevailing high temperature has thus far checked the spread of the disease. In these localities the following suggestions may be of value: 1. Some infection may be found in winter or early spring on the foliage of young and seedling trees. This probably will die out with the advent of warmer weather; but there is some danger of blossom infection, hence the diseased branches should be removed and burned. At least 2 inspections should be made, the first about 2 or 3 weeks prior to blossom time and the second 2 weeks later. This procedure has been tested and proved to be practical as well as efficient. 2. Inquiries have been made with respect to the advisability of planting pimento seedlings at the lower altitudes. This may be a worthwhile project, but should not be attempted until the pathogen, in its ability to spread, has come to an equilibrium with the temperature factor. This should take place within 2 to 3 years.

SUMMARY

A sudden outbreak of *Puccinia psidii* Wint. on the pimento tree, *Pimenta officinalis* L., occurred in Jamaica, B. W. I., in the spring of 1934. Within 2 years, the damage at the higher altitudes was so great that the products derived from the tree were almost a complete loss.

There appear to be 2 distinct strains of *Puccinia psidii* parasitizing pimento and rose apple, *Eugenia jambos* L., respectively, in Jamaica. *P. psidii* was originally described on *Psidium pomiferum* L., a variety of the common guava, *Psidium guajava* L. The latter tree has been reported as a host to *P. psidii* in Puerto Rico, but is apparently immune from the 2 strains of *P. psidii* found on pimento and rose apple, respectively. The bay-rum tree, *Pimento acris* Kostel, is susceptible to the strain parasitizing pimento, and the otaheite apple, *Eugenia malaccensis* L., is susceptible to the strain parasitizing rose apple.

Puccinia psidii attacks the expanding foliage, the inflorescence, and the succulent young twigs. Defoliation results in the development of new foliage that in turn becomes diseased. The fungus is perpetuated in Jamaica in the urediospore stage; teliospores were found, but no host for the haploid stage was recognized.

The incidence of the disease caused by *Puccinia psidii* on pimento is influenced primarily by 1, the relative abundance of susceptible new growth; 2, the existence of long periods of wet weather; 3, temperature. Temperature is the limiting factor that may determine the future of the pimento industry. A relatively large proportion of the pimento is reaped below an altitude of 1,000 feet, where *P. psidii* is either absent or doing no material harm because of the prevailing high temperature, which inhibits infection.

Use of fungicides to control the rust seems impracticable. Suggestions with respect to slight infections taking place at lower altitudes during winter and to the advisability of planting pimento seedlings at the lower altitudes have been given.

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THE RELATION OF CONCENTRATION OF FUNGICIDES AND BUD DEVELOPMENT TO CONTROL OF PEACH LEAF CURL[1]

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INTRODUCTION

The early discovery of the ease with which peach leaf curl (*Exoascus deformans* (Berk.) Fckl.) could be controlled by an application of a strong fungicide during the dormant period of the tree resulted in a rather general neglect of investigational effort to establish the minimum strengths of fungicides necessary to control the disease, and to determine the relation of bud development of the tree to infection and control. The accepted spray practices continue to give satisfactory results, but recommendations have not always been made with due consideration of the practical aspects of the possible monetary savings from the use of weaker sprays and of the fact that seasonal and orchard conditions frequently render spraying impossible until after the buds are active.

When used at the rate of 1 part in 8 of spray, lime sulphur gives good control of both San José scale (*Aspidiotus perniciosus* Comstock) and peach leaf curl, but the use of this material is not necessarily the most economical way to accomplish these results. For example, oil emulsion is a more efficient scalecide and Bordeaux mixture is usually a cheaper fungicide than lime sulphur. Furthermore, lime sulphur will control only San José scale and will not affect the terrapin scale (*Lecanium nigrofasciatum* Pergande) which is frequently troublesome in peach orchards. An oil spray must be used to control this insect and a fungicide may be added to make a complete insecticide-fungicide combination.

[1] Paper No. 87 from the Section of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

REVIEW OF LITERATURE

The life history of the peach leaf curl fungus was poorly understood for years, but it has recently been cleared up by the studies of Fitzpatrick (5) and Mix (9). The incomplete knowledge of the life history of the fungus led to the recommendation of control measures that were too dogmatic in nature.

The more or less general prevailing opinion is well expressed by Zeller (17) who writes, "The exact and very earliest time when this fungus infects the tiny leaves or twigs in the spring of the year is really unknown . . ." and that "Some infection takes place however as soon as the buds swell enough to cause the bud scales to slide over each other and to break the coating of wax which more or less seals the scales together, thus exposing the least bit of leaf tissue or green bases of the scales." Zeller recommends the application of a 6–6–50 Bordeaux mixture after the leaves have fallen and not later than December. This recommendation takes in consideration the problem of controlling California blight (*Coryneum beijerinckii*) as well as that of leaf curl. Heald (7, p. 467) states that spraying after the buds have swollen does not prevent the disease, and recommends that the orchardist "Spray *once*, either in the late fall, early winter or in the spring *before the buds start to expand*. . . ." Pierce (11) states "That most of the spores of *Exoascus deformans* enter upon the stage of germination at or about the time of pushing of the first leaf buds in the spring admits of little doubt. That is the time when the tissues of the peach leaf are most tender, and when their infection by curl is actually known to take place." He recommends that the tree be sprayed 2 or 3 weeks before growth begins, as the spores at that time are just beginning to germinate and are most susceptible to the action of fungicides. He did not consider the matter of later spraying.

Results of early work by various investigators indicated that spraying after the buds had swollen, or even made conspicuous growth, frequently gave successful control; but, despite this fact, the impression that spraying at such a time is always useless became widespread and rather generally accepted. Murrill (10) as early as 1900 recommended that the spray be applied " . . . about the first of April when the buds are beginning to swell." Laidlaw and Brittlebank (8) also recommended that the spray be applied "Just before or when the earliest buds are showing pink. . . ." This recommendation was based upon their investigations in which applications at such a time had been successful. Wallace and Whetzel (14) stated that an application to be effective, if not made before the buds had swollen, could still be made if applied before there was rain favorable to infection. A few years later Reddick and Toan (12) obtained good control in New York State rather late in April and when, in some orchards, the leaves were ½ inch long. No rains favorable to infection had occurred prior to that time, however, and when they set in on April 20, infections apparently developed, as control was not secured with applications made on April 26. Allen (1) in 1918 reported, fair control from applications made when the buds were

"at the pinking stage" although better control was obtained with earlier applications. A few years later Allen and Arthur (2) reported that results from spraying swollen buds were as good as those from buds sprayed while dormant. Weldon (15) obtained good control on the Simms variety in California, sprayed when "practically one half in bloom." Fitzpatrick (5) recently reported good control resulting from applications made when the leaves were fairly well out. He found time of infection to be correlated with periods of rainfall, confirming the observations of Wallace and Whetzel (14) and others.

The value of lime sulphur as a fungicide for the control of peach leaf curl was accidentally discovered in California at the time when this material was first being used as an insecticide to control San José scale. The spray used was necessarily quite concentrated, and, although it was recognized a number of years ago that somewhat weaker dilutions could be satisfactorily employed, no particular attempt was made to ascertain how much lime sulphur might be diluted and yet remain dependable. The tendency to recommend strong spray solutions has persisted in the absence of satisfactory experience with the weaker concentrations.

Pierce (11) used a lime sulphur made from a 5–5–45 formula, which, according to tests made during the current study, corresponds to approximately a 1 in 16 dilution of 32° Baumé lime sulphur, or about half the usual winter strength. Murrill (10) recommended a 6–4–50 Bordeaux mixture for dormant application and a 2–2–50 formula to be applied after the petal fall in wet seasons. Shortly afterward, Farrand (4, pp. 17–18) reported 2 pounds of copper sulphate in 50 gallons of water to make an effective spray. Laidlaw and Brittlebank (8) obtained poor control with lime sulphur, although Bordeaux mixture proved more satisfactory. Their best results were obtained with copper acetate and copper soda solutions. They recommended a 6–8–40 (6–8–50 U. S. standard) copper soda spray as being the most satisfactory, and yet economical. Wallace and Whetzel (14) in 1910 stated that "The fungicide used and the strength of it are of relatively little importance." They found a Bordeaux mixture as weak as 3–4–50 to control curl, and 32° lime sulphur diluted 1–20 to give fair control. Weldon (15), in reporting on comparative tests with dry and liquid lime sulphurs, stated that liquid lime sulphur, as weak as 1–30, and dry lime sulphur at 2 pounds in 30 gallons gave fair control of leaf curl, although somewhat better results were obtained with higher concentrations of each material. The dry and liquid forms proved equal in all tests when used at the rate of 2 pounds of dry material as the equivalent of 1 gallon of liquid lime sulphur. It should be noted that these applications were made when the trees were in almost semi-anthesis, much later than is generally recommended for leaf-curl control.

A year later Allen and Arthur (2) reported satisfactory control with lime sulphur in dilutions ranging from 1–7 to 1–12, with Bordeaux mixture at 4–6–22 (4–6–27½ U. S. standard) and with Burgundy mixture. Forma-

lin, used at the rate of 1½ ounces per gallon of spray, gave no control, nor did sulphur dust. The lime sulphur tested about 25° Baumé, which would make their dilutions correspond to approximately 1–9.7 and 1–16.7 of a 32° solution.

Wilson (16) was able to control leaf curl with fall applications of Bordeaux mixture as weak as 2–5–50, with lime sulphur 4–50 and with basic copper sulphate at 3 pounds in 50 gallons. Copper ammonium silicate (Coposil) at 3 pounds in 50 gallons of spray did not give as satisfactory control.

Stanley, Marcovitch and Andes (13) obtained control with wood creosote when used alone at 8 per cent strength and also when used at 1 per cent strength with 3 per cent oil emulsion.

Cation (3) states that lime sulphur 1–20 is the standard spray dilution used in Michigan, although some growers use a stronger spray. It is probable that very much the same situation exists in most peach-growing States. Zeller (17) recommends a 6–6–50 Bordeaux mixture for the control of both peach leaf curl and California blight.

METHODS AND MATERIALS

Peach trees known to be generally affected with leaf curl were selected for the spray experiments. The trees had in most instances been left entirely unsprayed for all or the greater part of the life of the orchard.

The plots where the various materials and dilutions of materials were under test were sprayed with a power-spray rig at 325 pounds pressure, multinozzle spray brooms being used for application. The individual trees or small plots were sprayed with a knapsack sprayer at 75 pounds pressure.

The plots were examined and the results determined after the trees were in full leaf and before the curl-affected leaves had begun to fall. The number of leaf clusters containing diseased leaves were counted, no attempt being made to count all of the disease-free leaves or leaf clusters. The trees selected for the experiments were for the most part fairly uniform in size, and the counts that were made gave a good indication of the degree of control obtained. Had the results been less clearly evident, it might have been necessary to count the actual number of infections per thousand leaves.

EXPERIMENTS IN 1933–34

Fall applications of various fungicides were made on 7-year-old Elberta peach trees in the Stephenson orchard near Winchester, Virginia, on November 21, 1933. The same treatments were repeated, with a few omissions, on an adjacent block on March 17, 1934. The plots were of 10 trees each. The fungicides employed, their rates of application, and the leaf-curl counts are given in table 1.

All materials used in 1933–34 gave satisfactory control of leaf curl at the several strengths or dilutions tested. The slight variation from plot to plot is not considered significant in view of the difficulty of completely

TABLE 1.—*Efficacy of various fungicides in the control of leaf curl, 1933–34 applications*

Plot No.	Materials and amounts used per 100 gallons	Diseased clusters per plot	
		Fall spray	Spring spray
1	Lime sulphur, 2 gallons	20	22
2	Lime sulphur, 4 gallons	11	0
3	Lime sulphur, 6 gallons	0	1
4	Lime sulphur, 9 gallons	1	0
5	Lime sulphur, 12 gallons	0	9
6	Dry lime sulphur, 16 pounds	0	Omitted
7	Dritomic sulphur, 16 pounds	58	2
8	Dry flotation sulphur, 16 pounds	4	Omitted
9	Soluble sulphur, 8 pounds	0	6
10	Soluble sulphur, 16 pounds	0	Omitted
11	Kolofog, 16 pounds	11	16
12	[a]Bordeaux, 2– 4–100 formula	57	30
13	[a]Bordeaux, 4– 8–100 formula	6	0
14	[a]Bordeaux, 6–12–100 formula	1	0
15	[a]Bordeaux, 8–16–100 formula	0	Omitted
16	Check, nonsprayed	1859	1858

[a] All Bordeaux mixture was prepared with a special grade of hydrated spray lime and in accordance with common practice.

covering all of the surface of 10 trees, and of the possible occasional infection resulting from conidia carried by wind or rain from adjacent nonsprayed trees. The plots sprayed with 2–4–100 Bordeaux showed slightly more leaf curl for both the fall and spring treatments than did those sprayed with the stronger combinations. The difference was only slight, however, and was but 2.4 per cent of the infection on the checks.

EXPERIMENTS IN 1935

The further testing of numerous fungicides at varying strengths was not continued in 1935 because of the favorable results obtained in the 1933–34 season and the decision of the owner of the orchard in which the experiments were being conducted to do the spraying himself.

Appreciation of the fact that only a very few growers complete, and that many frequently do not begin the leaf-curl spray prior to the swelling of the buds, led to the trial of a delayed spray application in 1935, which in timing paralleled that of many growers. Fifteen trees were sprayed in the Stryke orchard, a small home planting near Winchester. One spray was applied on March 18, 1935, when the young leaves were protruding about $\frac{1}{8}$ inch from the buds. There was a rather uniformly distributed rainfall of 1.64 inches for the 3-day period, March 11, 12, and 13, four days before the spray was applied. Since the peach buds were conspicuously swollen at the time these rains occurred, it would seem that there was ample opportunity for leaf-curl infection to take place before the spray was applied on March 18. Standard 32° lime sulphur diluted 1–40 was used, since it was considered sufficiently effective to obtain control if infection had not already taken place. A stronger solution might have caused serious injury to the buds.

The 1935 experiment gave a striking demonstration of leaf-curl control, the sprayed trees showing an average of only 130 infected leaf clusters per tree, whereas there were 2,825 diseased clusters on the check tree.

EXPERIMENT IN 1936

A further check on the possibility of controlling leaf curl by spraying after the buds had swollen conspicuously or had made considerable growth, was sought in 1936. Spray applications were planned to begin while the buds were yet dormant and to continue at frequent intervals until the immediate pre-blossom stage.

A row of Elberta trees were selected in the Schmidt orchard near Winchester, an orchard that had been severely infested with leaf curl for several seasons. The trees varied in size and from 1 to 3 trees per plot, with most plots containing only 2 trees. All plots, with the exception of the check, were sprayed with lime sulphur 1–80. A knapsack sprayer was used, because soil conditions made impossible the use of a power rig (Table 2).

The results in 1936 were less conclusive than those of other years, although all applications were clearly beneficial. The first two applications were made with 16° Baumé lime sulphur through mistake. The later applications were made with 32° lime sulphur diluted to give the same strength, or 1 part in 80, so that the treatments would be uniform.

TABLE 2.—*Control of peach leaf curl obtained from delayed applications of lime sulphur in 1936*

Plot No.	Date sprayed	Stage of bud development	Number of diseased leaf clusters
1	March 5	Dormant	16
2	March 23	Swollen, silver tip	7
3	March 27	Leaves out ⅛ to ¼ inch No color in blossom buds	21
4	April 1	Leaves out ¼ to ½ inch	28
5	April 4	Leaves out ¼ to ¾ inch Pink in blossom buds	34
6	April 8	Leaves out ½ to 1½ inches Some blossoms open[a]	31
7	Check, no spray		92

[a] The peaches were in full bloom on April 14.

Rainfall throughout the duration of the experiment in 1936 was frequent and prolonged enough to give excellent opportunity for infection. Rain fell on 9 days between March 5 and 23; on 3 days between March 23 and 27; on 1 day between March 27 and April 1; on 2 days between April 1 and 4; on 1 day between April 4 and 8. There was a total of 9.09 inches of precipitation from March 5 to April 8.

EXPERIMENTS IN 1937

Eleven fungicides in one or more dilutions were tested in 1937 to check further their effectiveness in the control of peach leaf curl; but, since there

was not a significant amount of disease on the nonsprayed checks, no counts were made. No curl was observed on the trees in any of the sprayed plots. Additional information, however, was obtained on the control of the disease resulting from spray applied after conspicuous bud development. These experiments were located in a different orchard.

FIG. 1. Stage of peach bud development on dates of 1937-delayed-spray applications. A. Dormant application, March 3. B. Second application, March 9. C. Third application, March 12. D. Fourth application, March 23. E. Fifth and final application, April 1. F. Nonsprayed peach branches showing severe leaf curl infection. G. Noninfected branch sprayed with 1-40 lime sulphur on March 23, 1937.

A number of old neglected trees in the Virginia Valley orchard near Stephens City, Virginia, were selected for the delayed fungicide applica-

tions. Each plot consisted of 2 trees, the checks being located between the sprayed plots. Lime sulphur 1–40 was applied with a knapsack sprayer, as previously. The first application was made while the buds were dormant, and successive applications were made at intervals as the buds developed. Shoots were cut off at the time of each spray and photographed (Fig. 1). The dates of the spray applications and the amount of leaf curl that subsequently developed are given in table 3.

TABLE 3.—*Peach leaf curl counts on Elberta trees sprayed at intervals from the dormant period until the first blossoms opened. Stephens City, Virginia, 1937*

Plot No.	Date sprayed	Number of diseased leaf clusters	Plot No.	Date sprayed	Number of diseased leaf clusters
5	April 1	149	1	March 3	72
6	Check, no spray	4590	2	March 9	18
7	Check, no spray	3370	3	March 12	30
			4	March 23	111

The degrees of control obtained with the delayed applications seem to indicate either that very little infection had taken place prior to April 1 or that the lime sulphur was able to kill or "burn out" the young infections. The rainfall for March was light, being only 1.15 inches. Two showers totalling 0.42 inch fell after March 15, the remainder of the precipitation falling earlier in the month in the form of snow.

DISCUSSIONS AND CONCLUSIONS

The ability of relatively weak fungicides or of dilute concentrations of strong fungicides to control peach leaf curl has been indicated from time to time in numerous scattered reports. That such observations are valid is supported by the results obtained in orchard experiments and reported in this paper. No difficulty was experienced in controlling the disease with Bordeaux mixture as weak as 2–4–100, with lime sulphur diluted 1–50 or with proprietary wettable sulphurs at 16 pounds per 100 gallons. Soluble sulphur (dry sodium polysulphides) gave excellent control at 8 pounds per 100 gallons of spray, the weakest strength tested. Lime sulphur in a 1–40 dilution was always effective in leaf curl control (Fig. 1, G) when used in spraying the buds in progressive stages of development. Lime sulphur 1–80 noticeably reduced the amount of leaf-curl infection in a limited trial, but was not completely effective.

There is no doubt that the strengths of fungicides generally recommended for control of peach leaf curl are effective. Lime sulphur at 1–8 will also control San José scale, but frequently its use at this concentration is not the most economical way to control leaf curl and San José scale. Lime sulphur and Bordeaux mixture at the concentrations normally used as summer sprays on apple will control peach leaf curl in Virginia, and there seems to be no valid reason for using them in stronger concentrations

Either of these fungicides can be used in combination with oil emulsion to provide a combination fungicide-insecticide spray, except where soap has been used as the emulsifier for the oil. Bordeaux mixture may be used as an emulsifier where it is desired to prepare an emulsion in the spray tank, and it, together with the oil emulsion, provides the lowest cost combination spray.

Several investigators have obtained satisfactory control of leaf curl by spraying after the buds were conspicuously swollen or even after the young leaves had protruded. The results reported here substantiate these observations and, contrary to the common assumption, clearly indicate that the end of the period for the control of peach leaf curl is not necessarily coincident with the first active swelling of the buds. Good control was obtained repeatedly in these experiments with sprays applied after swelling and growth of the buds had begun and until the first blossoms were opening. No sprays were applied later than this stage of development. It is quite possible that seasonal conditions may occur in which control could not be obtained after evident bud activity, but this does not alter the fact that in several seasons, when leaf curl was generally prevalent, control was obtained with delayed spray applications. During these years, weather conditions during the period of bud development were favorable for infection.

The possible ability of liquid lime sulphur to burn out young leaf-curl infections was suggested by Fitzpatrick (5), and its ability to accomplish such action in the case of apple scab (*Venturia inaequalis*) infections as much as 72 hours old was demonstrated by Hamilton (6). There is a possibility that lime sulphur may act in the same manner against young leaf-curl infections when delayed applications are made. Some of the early investigators reported unsatisfactory results from sprays applied after bud activity had begun; and, as a matter of safety in general practice, it is no doubt best to apply the leaf-curl spray either in the fall or as early in the spring as possible, but at the same time it should be recognized that many growers cannot or will not complete the spray within the time limits normally set. Seasonal conditions and individual problems frequently delay applications beyond the time commonly advised. The results of other investigators, which have been reviewed here, together with the new data presented, indicate that where such a spray cannot be applied in accordance with the normal schedule, results from later applications are satisfactory frequently enough to justify completing a spray at any time prior to blossoming. A reasonably mild or weak fungicide should be used as a matter of safety at this time. Exception is not being taken to present recommendations, but it is suggested that recommendations for leaf-curl control should be revised so as to recognize the benefit that may be derived from delayed spray applications.

SUMMARY

Peach leaf curl was controlled with fungicides in much weaker concentrations than are usually recommended. Lime sulphur 1–50, Bordeaux mix-

ture 2–4–100, and wettable sulphurs at 16 pounds in 100 gallons have all given effective control.

Good control of leaf curl was obtained with sprays applied after the leaves were protruding as much as one inch from the buds. Lime sulphur 1–40 was used in the delayed applications.

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RELATION OF STOMATA TO INFECTION OF PINUS STROBUS BY CRONARTIUM RIBICOLA

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INTRODUCTION

While studying the environmental factors influencing infection of 5-needle pines by *Cronartium ribicola* Fischer, a detailed study was made of the stomata of needles of *Pinus strobus* L. because Clinton and McCormick (3) stated that the germ tubes from sporidia of *C. ribicola* enter the needles by way of the stomata. The presence of substomatal vesicles beneath the stomata in young needle infections led them to conclude that fungal invasion occurs through the stomata.[2] They failed, however, to demonstrate any direct connection between the substomatal vesicles and sporidia on the exterior of the needles. They attributed this failure to the fact that "not sufficiently young infections, those less than a week old, have been examined, and the exceedingly great difficulty in locating points of infection, since there is no external evidence to guide one within three or four weeks after this takes place."

An attempt was made to determine the nature and development of the substomatal vesicles and to gather various stomatal statistics that might possibly affect the entrance of germ tubes of sporidia of *Cronartium ribicola* through stomata. The results are presented in this paper.

METHODS

The structure and periodic movements of the stomata were studied at the Charles Lathrop Pack Demonstration Forest, Warrensburg, New York, during the summers of 1931 and 1932. The first summer was spent largely in developing a satisfactory method of measuring stomatal activity. The data secured in 1931 agreed with those of the following season, but the results recorded in this paper were obtained from June 20 to August 20, 1932. The studies concerned with the mode of needle penetration were done during the summer of 1933 at Warrensburg and the autumns of 1934 and 1935 at the New York State College of Forestry, Syracuse, New York.

The needle samples for the studies of stomatal opening and closing were taken from trees subjected to the several growing conditions subsequently described in this paper. It was deemed necessary to study the stomata in needles of young pines growing in pots, as well as those of older pines grow-

[1] The investigations here reported were made by the writer while employed as Agent in the Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the New York State College of Forestry at Syracuse, New York. The stomatal measurements were made by Mr. J. L. Lowe under the direction of the writer. Mr. J. S. Kring prepared part of the materials used in the study of needle penetration.

[2] Drs. Clinton and McCormick most graciously permitted the writer to study their slides on several occasions and very kindly discussed their interpretations in detail.

ing under natural conditions, because the study here reported was only one phase of another investigation. The potted pines were 4 years old, having been grown in seed beds for 2 years, in transplant beds for 2 years, and planted in pots during April, 1932, after which they were placed in the open and subjected to the prevailing weather conditions.

In studying the opening and closing of stomata it was impossible to use an ordinary microscope and direct light, or a vertical illuminator, such as an Ultropaque, in order to view the stomata in living needles, because the stomatal pits[3] are filled with a substance which obscures the guard cells and stomatal pores. The porometer method (4; 6; 9, p. 328) of measuring stomatal opening did not lend itself readily to the work with pine needles. Hence it was necessary to work with preserved material and to choose a method whereby the guard cells were quickly killed and fixed in position and by which the obstructing substance in the stomatal pits was quickly removed. The alcohol-fixation method, suggested by Lloyd (7) and by Loftfield (8) and recently severely criticized by Hartsuijker (4) and by Oppenheimer (10), gave definite and satisfactory results with the needles of white pine, and was, therefore, used in this study.

Five needles each of 1930, 1931, and 1932 origin were removed from each of the selected trees at the beginning of every hour during 24-hour periods. A portion of the epidermis was immediately stripped from each needle and immersed in absolute alcohol. Within the hour the strips of epidermis were mounted in absolute alcohol and examined microscopically. After examining and comparing the stomata from the 5 different needles of each needle age, 25 stomata from a typical needle were measured and recorded for each of the needle ages. When the pores were 0.5 μ or more in width they were considered "open" and were measured with a calibrated ocular micrometer. Pore-openings less than 0.5 μ in width were approximated and are recorded in this paper as "partly open."

The data on stomatal structure were obtained largely from free-hand sections of living needles and, to some extent, from stained sections made into permanent mounts.

In order to study the penetration of the needles by the germ tubes of sporidia, living needles still intact on the trees were inoculated. Young potted pines were used, as well as naturally established trees. The needles were inoculated by suspending small sections of telium-bearing *Ribes nigrum* leaves above marked portions of pine needles. Only those infected *Ribes* leaves known to be producing sporidia at the time were used. When potted pines were used, they were placed in a damp chamber under conditions favoring the germination of sporidia. On trees growing in the open, the needles were inoculated in the early evening or during periods of cool, rainy weather. Sufficient needles were inoculated at one time to permit one or more samples to be taken every 6 hours, up to and including 72 hours. A few needles

[3] Throughout this paper the urn-shaped chamber formed by the accessory epidermal cells above and leading to the guard cells is designated as the stomatal pit.

were removed from trees, inoculated, and placed on wet paper in Petri dishes kept in a cooler at about 53° F. The samples were killed and fixed in chromo-acetic solution contained in appropriately labeled vials. Later, they were embedded in paraffin, sectioned, stained, and mounted.

ARRANGEMENT AND STRUCTURE OF STOMATA

The stomata of *Pinus strobus* are arranged in several rows extending longitudinally along the 2 flat sides (ventral surface) of each needle (Fig. 1).

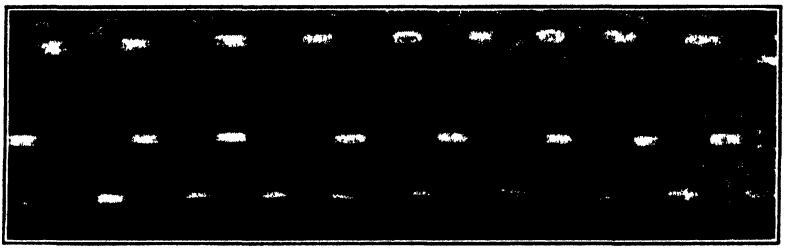

FIG. 1. Arrangement of stomata in needles of *Pinus strobus*. × about 120. (Photograph by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.)

They are sunken below the epidermis and each communicates with the needle surface by means of an outer stomatal chamber or pit that, in mature needles, is plugged with a substance stated to be wax or a mixture of wax and resin (2, p. 214; 11; 12). In 1932, this material appeared in the stomatal pits above the sunken guard cells of the current-season needles about July 13, when the needles extended approximately 2.5 centimeters beyond the enclosing sheath and were somewhat over one-third the average length of the mature needles at the end of the summer period. As previously stated, it was these plugs that made it impossible to see the stomatal pores in living needles and thus necessitated the removal of strips of the epidermis and their immersion in absolute alcohol. The alcohol not only fixed the guard cells until the material could be examined, but it partially removed the waxy deposit and exposed the stomatal openings.

The first study was made in order to determine as nearly as possible the greatest distance from a stoma that a sporidium could be when it came to rest upon the ventral surface of a mature needle. This was considered to be the distance from the edge of a needle to a stomatal pore in the nearest row of stomata at the center of the long axis of the needle. The average distance between the pore openings of adjoining rows of stomata and the average distance from stomatal opening to stomatal opening of consecutive stomata within the same row also were determined. These distances were found to be approximately the same on needles taken from potted pines, from young pines growing in the open, and from older pines in the forest; therefore the data are combined in table 1.

The rows of stomata are unequally spaced on the needle surface, and varied from as little as 47 μ apart to as much as 201 μ. Within the rows,

TABLE 1.—*Stomatal statistics for needles of Pinus strobus*

Item	Needles of indicated origin					
	1930		1931		1932	
	Number measured	μ	Number measured	μ	Number measured	μ
Distance between edge of needle surface and pore of nearest row of stomata	75	165	75	161	75	141
Distance between pores of one row of stomata and the next	75	89	75	95	75	88
Distance between pores of consecutive stomata within a row	75	33	75	32	75	32
Depth of:						
Pit	62	15.2	75	14.7	69	14.5
Pore	62	10.8	75	11.2	69	10.9
Pit and pore	62	26.0	75	25.9	69	25.4

most of the stomata are so close to one another as to nearly touch, thus making the distance between the pores almost equal. The distance from the outer edge of the needle surface to the nearest pore varied but was rarely over 185 μ. In the 1930 and 1931 needles the distance was rarely less than 140 μ. The 1932 needles had apparently not attained their maximum width at the time they were examined, although they appeared to be mature. The maximum and minimum distances from the edge of the 1932 needles to the nearest pore were 192 μ and 102 μ, respectively.

In surface view each stoma appears as a pair of bean-shape cells with a depression between them (Fig. 2, A). These bean-shape cells are accessory epidermal cells, superimposed upon guard cells of similar shape (Fig. 2, B), the walls of which may be seen at the bottom of the depression or pit formed by the 2 accessory cells. The stomatal pore is considered as the opening between the 2 guard cells, and its depth the distance from the upper walls of the guard cells to an imaginary line drawn across the top of the substomatal chamber between and touching the bases of the 2 guard cells. In cross section the guard cells differ in appearance, depending on the location of the cut through the stoma (Fig. 2, D, E, F). Near the end of a stoma, beyond the edge of the pore, they have an additional wall thickness appearing as flange-like appendages (Fig. 2, F), so placed as to touch each other, and apparently are never drawn apart to enlarge the pore. At the edge of the pore (Fig. 2, E) they also appear to have flange-like appendages, but these are not observed in sections made at or near the center of the pore (Fig. 2, D).

Measurements of the depth of the stomatal pit and the pore were made, since this is the minimum distance a germ tube would have to grow in order to reach the interior of a needle. To this end needles were cross sectioned and only those stomata, sectioned at approximately the center of the pore, were measured. Again, it was discovered that the measurements were almost ex-

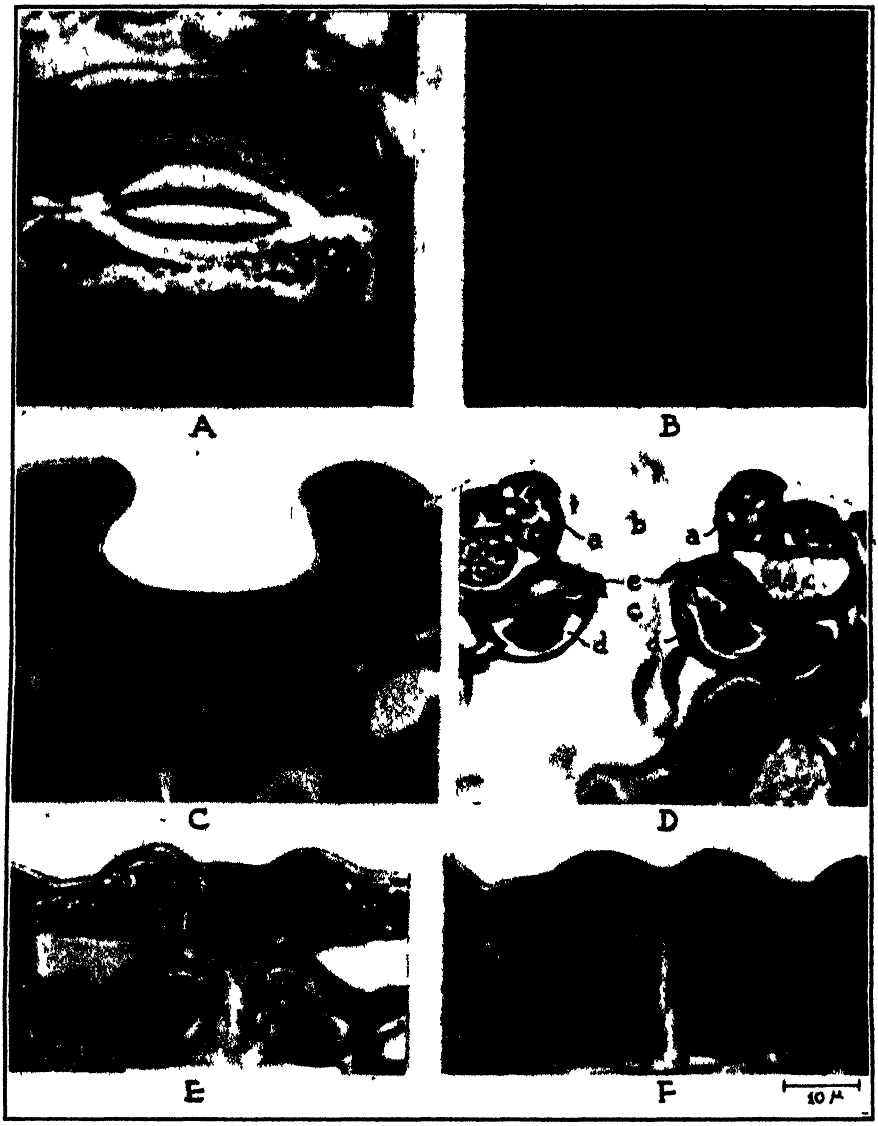

FIG. 2. Stomata of *Pinus strobus*. A. Surface view of a stoma showing 2 bean shape epidermal cells above the guard cells. B. Surface view of the guard cells after the accessory cells have been removed. C. The side of a guard cell facing the pore. Note the large nucleus and the thick cell walls. D. Cross section of a stoma at center of pore: a, accessory epidermal cells; b, outer stomatal pit; c, pore (the processes involved in the preparation of permanent mounts affect the cells in such a way that the stomata are open wider than they actually occur in living needles); d, guard cells; e, beak-like projections of guard cells. E. Cross section of stoma near end of the pore showing a stomatal plug, (f) and flange-like appendages (g) on the inner walls of the guard cells. F. Cross section of a stoma near the end of the guard cells away from the pore where the thickened walls remain pressed together.

actly the same for all the needles, regardless of their source; hence, the data are recorded in table 1 according to needle age only. There was relatively

little difference in the depth of the stomatal pits and pores in mature needles of like and different ages.

STOMATAL MOVEMENTS

The extreme edge of the walls of the guard cells at the pore appears in cross section as a beak with the upper or outer wall extending beyond the lower or inner wall (Fig. 2, D). The opening and closing of stomata of eastern white pine is in part the result of these beak-like extensions moving toward and away from each other. When a stoma is closed it is possible to observe in properly prepared mounts these extensions closely pressed together. In open stomata they seldom withdraw from each other more than 1.5 μ. Rarely are all the stomata of a needle open or shut at one time, although the majority may be.

As stated earlier in this paper, every hour during certain 24-hour periods, strips of epidermis from 5 living needles of each of 3 needle-ages were immersed in absolute alcohol, examined, and microscopically compared. In general, it was found that, for a particular needle age, the width of the stomatal pores on 1 needle were representative of the 5 needles. Twenty-five stomata from one of the 5 needles of each needle age were measured and recorded as the widths of the pores for a needle of a particular needle age for a particular hour.

At the time of maximum opening a few stomata opened as wide as 1.5 μ, very rarely to 3 μ; the majority of the pores averaged approximately 0.5 μ.

Studies of stomatal activity were made on pines of different ages, on different sites, and subjected to natural and artificial environments as follows:

1. A 12-year-old pine, growing in the open in sandy soil on the edge of a swamp.
2. A forest tree about 30 years old growing on an eastern slope in coarse gravel loam.
3. An 8-year-old pine, growing in sand in the open. Comparative studies were made on rainy days and on clear, relatively dry days.
4. A potted pine kept on the same site as the 30-year-old forest tree. Studies were made on these two trees during the same hours and days.
5. Three potted pines kept under damp-chamber conditions where the needles were continually moist. The temperature ranged between 50° and 60° F. and there was almost no light.

In addition, several forest trees approximately 50 years old growing on a rocky slope and several trees approximately 30 years old growing in a swamp were studied at various times during the summer of 1932 for the purpose of comparison with the more exact studies of the trees listed.

During the work in 1932, over 24,000 stomata were studied and equally as many in 1931 in preparation for the work of 1932.

Although there were some differences in the percentage of open stomata between the trees for specific hours, they were not significant. A large per-

centage of the stomata were open to some degree during every hour of the day, although the largest number was open or partly open between approximately 10 a.m. and 2 a.m. daily. This is shown in table 2, which represents

TABLE 2.—*Stomatal activity of a 12-year-old white pine from June 20 to July 22 and August 15 to August 20, 1932*[a]

Time		Condition of stomata					
		Current-season needles			Year-old needles		
		Closed	Partly open	Open	Closed	Partly open	Open
a.m.	*p.m.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8		51	34	15	64	34	2
10		40	34	26	56	36	8
12		35	38	27	51	38	11
	2	28	28	44	54	43	3
	4	26	28	46	48	41	11
	6	20	36	44	47	48	5
	8	25	28	47	42	53	5
	10	29	40	31	59	41	0[b]
	12	24	44	32	63	37	0
2		32	35	33	63	37	0
4		52	29	19	73	23	4
6		55	35	10	70	30	0

[a] Data based on 450 stomata on 18 needles per needle age for each hour indicated in the table.

[b] Although in the microscopic field all of the stomata may have been closed, there were always occasional stomata open on a needle.

the daily stomatal activity of a 12-year-old white pine between June 20 and August 20, 1932. The same stomatal activity occurred in the 8-year-old pine, both during periods of clear warm weather and cooler periods of rain. The stomata behaved the same on the forest trees as on potted pines subjected to natural meteorological conditions. In the damp chamber, however, nearly all the stomata on the potted pines were open to some degree between 8 a.m. and 8 p.m. and the majority were partly open during the remaining hours.

The stomata in the current-season needles were more active than those in the needles produced the previous year. The data for the needles of 1930 origin were taken but are not recorded in table 2, since they nearly parallel those for the stomata of the 1931 needles.

NEEDLE PENETRATION

Somewhat over 22,000 cross sections of inoculated needles were made into permanent mounts and studied for needle penetration by *Cronartium ribicola*. Approximately 2500 of the sections showed germinating sporidia present. In addition many temporary mounts were made and studied. The sporidia were in all stages of germination, some with germ tubes just appearing, others with tubes as long as 33 μ. Sporidia were found to have fallen not only near stomata, but many times directly upon the waxy plugs that filled the stomatal pits. None of the germ tubes were found to have grown through a stomatal pore into a needle; indeed, germ tubes of sporidia resting

directly above the stomata were observed growing along the epidermal surface away from the stomata. Commonly, the germ tubes from sporidia lying near stomata grew directly across them without any trace of downward growth toward the stomatal pores. This was true of germ tubes from both primary and secondary sporidia[4]: the latter being recognized by their placement near the ends of the sterigma-like germ tubes (5, fig. 4, p. 18) of the primary sporidia. In 7 instances, germ tubes growing from sporidia were found to penetrate the epidermal cells directly, some hyphae extending as deep as the hypodermal layer.[5] Penetration occurred through both the dorsal and ventral needle surfaces. The germ tubes entered the epidermal cells in 2 ways: 1. They retained their normal width as they passed through the cell walls. 2. They formed a fine, needle-like extension at their tip. No appressorium was present in either type of penetration, nor were any swellings of the germ tubes observed either within or outside the needle.

Although the writer failed to find substomatal vesicles associated with the phenomena of penetration, he observed vesicles present in established infections. Substomatal vesicles were present in living infected needles examined as long as 11 to 14½ months after the date of inoculation. Not only were substomatal vesicles present in the discolored spot, which is thought to denote the point of initial establishment of the fungus in a needle, but a substomatal vesicle was discovered in a section of a needle taken at a distance of somewhat over 3 mm. beyond the margin of the discolored spot and toward the base of the needle. This infection was 14½ months old; the mycelium had grown from the point of initial infection downward through the needle into the bark.

STOMATAL CHARACTERISTICS AS RELATED TO PINE INFECTION

It was not determined to what extent the waxy plugs in the outer stomatal chambers influence infection. These plugs appeared to consist of a mass of separate particles between which it seems possible that hyphae may force their way. The plugs normally filled the outer chambers but did not extend into the pores. Sporidia germinating upon living pine needles were examined by means of an Ultropaque. The germ tubes were found to be growing on the epidermis without any apparent regard for the stomata. The young hyphae were seen to grow between stomata as well as directly across and beyond them. Occasionally, hyphae were traced to stomata where they appeared to terminate, but it was impossible to see whether they grew downward into the pores. As previously stated, cross sections through such needles failed to disclose hyphae in the stomatal pit or pores.

[4] The production of secondary sporidia by primary sporidia of *Cronartium ribicola* appears to be a normal process and not the result of unnatural environment. Upon needlss of white pine they produce typical hyphae and as Spaulding and Gravatt (13) state they are no doubt "potentially dangerous to pines for much longer periods than the longevity of the primary sporidia would indicate."

[5] The examples of direct penetration have been examined by J. S. Boyce, G. G. Hahn, J. R. Hansbrough, and P. Spaulding of the Division of Forest Pathology, U. S. Dept. of Agriculture. The slides are now deposited in the Dept. of Forest Botany and Pathology at the New York State College of Forestry, Syracuse, N. Y.

For some time the writer has been studying the germination of sporidia of *Cronartium ribicola* placed on solid water agar and subjected to various temperatures (5). It was found that when freshly cast sporidia were kept at temperatures of 50° to 60° F. on solid water agar the following growth resulted at the end of the hours indicated in parentheses: No germination (2); young germ tubes on primary sporidia (4); secondary sporidia present and a few germinating (6); germ tubes of secondary sporidia averaging 10 to 11 μ (8); germ tubes mostly 20 to 40 μ (10); germ tubes mostly 20 to 50 μ (12 and 14); germ tubes mostly 20 to 60 μ (16); germ tubes mostly 40 to 100 μ (18 and 20); germ tubes mostly 40 to 106 μ (22). By means of an Ultropaque it was ascertained that, under similar conditions of moisture and temperature, the germ tubes from sporidia elongate at approximately the same rate on pine needles as on solid water agar. Table 1 shows that the average distance from the outer surface of a needle to the bottom of a stomatal pore is approximately 26 μ. If germ tubes enter a needle by way of the stomata, at least 10 hours must elapse following deposition of primary sporidia on the needles before the germ tubes can grow through the pit and pore, and then only provided the sporidia come to rest directly over the stoma. It has been observed (5) that at 50° to 60° F. primary sporidia tend to produce secondary sporidia; whereas, under the same conditions of temperature and humidity, secondary sporidia germinate and form true hyphae. Six hours after planting primary sporidia upon water agar they produced secondary sporidia that, 4 hours later, had germ tubes from 20 to 40 μ long. Therefore, if secondary sporidia are carried to a needle and fall upon the stomata, a germ tube may grow the distance from the needle surface to a substomatal chamber within a minimum of 4 hours. Hence, if the fungus invades the needles by way of the stomata, it is obvious that for needle infection to occur in a relatively short interval of favorable weather, sporidia must come to rest in the immediate vicinity of the stomata.

It is difficult to determine just what relation, if any, stomatal activity may have to needle penetration. A relatively large number of stomata were open to some degree at all times and would thus permit fungal invasion at any hour of the day. Most of the stomata were open between approximately 10 a.m. and 2 a.m. In the course of certain studies at Warrensburg, New York, in 1930, 1931, and 1932, a series of potted pines was exposed at stated intervals to natural infection by *Cronartium ribicola*. Equal numbers of pines were exposed daily from 8 a.m. to 8 p.m. and from 8 p.m. to 8 a.m.[6] One hundred and forty-two infections developed on 75 of the trees. Twenty-two of the infections were on trees subjected to inoculation between 8 a.m. and 8 p.m.; 120 infections occurred on those trees exposed between the hours of 8 p.m. and 8 a.m. Another series of trees was inoculated at the same time and in the same location as the first series but, following inoculation, the trees were placed for 36 hours in a damp chamber under conditions favorable to spore germination. In the second series, 151 infections developed upon

[6] These hours correspond approximately to periods of daylight and darkness.

77 trees; 4 infections occurring upon trees exposed between 8 a.m. and 8 p.m. and 147 on trees exposed between 8 p.m. and 8 a.m. Although several factors were involved that would definitely influence the amount of resulting infection, nevertheless, the fact remains that the greatest number of infections occurred on those pines subjected to inoculation when the fewest stomata were open. The fact that the number of open stomata and their size do not appear greatly to influence the amount of infection is demonstrated better in another experiment. During 1930, 1931, 1932, and 1933 a series of pines was subjected to natural inoculation by the blister-rust fungus. One hundred and ten trees became infected. At the time these 110 trees were subjected to inoculation, the year-old needles measured 173,260 linear inches; the current-season needles measured 157,310 linear inches. Through the year-old needles 152 infections occurred or 1 infection to approximately every 1100 linear inches of needles; through the current season needles 38 infections took place or 1 infection to approximately every 4100 linear inches of needles. In view of the fact that table 2 shows a greater percentage of stomata open in the current-season needles than in the year-old needles and that a greater percentage are open wider, it can be assumed that open stomata have relatively little effect upon the ability of the fungus to become established in the needles of white pine.

The fact that hyphae of *Cronartium ribicola* have been found to enter pine needles by direct penetration of the epidermal cells does not eliminate the possibility of entrance by way of the stomata. It is known that germ tubes from sporidia of rust fungi do penetrate their hosts in both ways but that direct penetration is more common than entrance through stomata (1). The fact that many sections with germinating sporidia upon them disclosed evidence only of direct penetration suggests that this may be the more common method with the blister-rust fungus.

The nature and significance of substomatal vesicles in needles of white pine are still to be determined. Arthur (1, p. 230), in referring to substomatal vesicles, states that "With the development of the hyphae the substomatal vesicle sometimes becomes divided by one or more septa, or it collapses and disappears, depending upon the species." In white pine needles substomatal vesicles are present in living needles a year or more after inoculation and are found even outside the discolored spot that is supposed to indicate the place of entrance of the germ tube and the establishment of infection. If substomatal vesicles in white pine needles are swellings of such delicate structures as young germ tubes, they would not be expected to persist for months after their formation, and if they mark the point of entrance they should be found only in the needle spots.

SUMMARY

The direct penetration of the epidermal cells by germ tubes from sporidia of the blister-rust fungus is reported for the first time.

It seems probable that stomatal activity has very little significance in the infection of *Pinus strobus* by *Cronartium ribicola.*

Stomata of *Pinus strobus* are arranged in rows on the ventral surface of the needles.

The average distance between the pores in contiguous rows of stomata was found to range from 88 to 95 μ; between the pores of consecutive stomata within a row, the average distance was 32 μ; and the distance in a straight line from any point on the margin of a stoma-bearing surface of a mature needle to the nearest pore averaged between 161 and 165 μ.

A stoma is opened and closed, in part, by the movement of the beak-like projections of the guard-cell walls.

Considering the depth of the stomatal pit and pore, it is probable that a minimum of 10 hours of favorable weather must elapse, following inoculation by primary sporidia of *Cronartium ribicola,* before a hypha can elongate sufficiently to come in contact with the mesophyll tissue by way of a stoma. In case of inoculation with secondary sporidia, the time may be shortened to 4 hours.

The greater percentage of the stomata were open between 10 a.m. and 2 a.m. This periodic opening occurred similarly in potted pines and pines growing under natural conditions, during periods of precipitation as well as intervals of clear weather with low relative humidity.

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INTERRELATION OF TAKE-ALL LESIONS ON THE CROWNS, CULMS, AND ROOTS OF WHEAT PLANTS[1]

HURLEY FELLOWS

(Accepted for publication Sept. 23, 1937)

INTRODUCTION

When a wheat plant is attacked by take-all, the presence and relative severity of the disease on various parts of the plant are of interest, especially in estimating the degree of injury.

The present study was undertaken to determine the interrelations of the occurrence of take-all lesions, caused by *Ophiobolus graminis* Sacc., on the culms, crowns, and roots of wheat plants and the degree of severity of the disease on these structures.

METHOD

Data were obtained from wheat plants grown in soil that was naturally infested with *Ophiobolus graminis*. The plants were grown in pots or boxes in the greenhouse from 1928 to 1934, inclusive. Many conditions with respect to moisture, temperature, and the use of soil amendments and fertilizers and various chemicals not in the fertilizer class were represented. Results are not presented individually for each experiment, since, for the purpose at hand, they were substantially alike. The data were taken after heading but before maturity. The crown, culm, and roots of each plant were examined and placed in 1 of 5 categories according to severity of the lesions showing; viz., slight, slight-medium, medium, medium-bad, and bad. The number of diseased crowns, culms, and roots also was recorded, as were certain other data, such as height of plants, number of culms and plants, number of dead and living plants, number of plants that tillered, and number of heads formed. The data from each pot or box were recorded separately. Those from each pot or box are here designated a "test."

RESULTS

The data are presented in two different ways for clarity and completeness. One method consists of placing the plants in the 5 different groups, according to the percentage of diseased crowns. The average percentage of crowns, culms, and roots diseased; the average severity of the disease on the crown, culms, and roots; the percentage of roots lost; the percentage of plants dead; the average height; the percentage of culms heading; the percentage of plants tillering; and the total number of tests and plants involved for each group were determined (Table 1).

[1] Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Botany, Kansas Agricultural Experiment Station. Contribution No. 354 from the Department of Botany, Kansas Agricultural Experiment Station.

TABLE 1.—*Average occurrence and severity of take-all on crowns, culms, and roots of five groups of Kanred wheat plants grown in the greenhouse under several conditions, the data being grouped according to percentage classes of diseased crowns*

Percentage classes of diseased crowns	Number of:		Average height of plants	Plants tillering	Culms heading	Plants dead
	Tests	Plants				
			Inches	*Per cent*	*Per cent*	*Per cent*
0.0	43	197	33.6	66.9	53.1	0.0
0.1 to 50	66	557	26.9	50.0	47.7	4.7
50.1 to 85	69	718	29.0	42.5	43.5	1.0
85.1 to 96	49	727	23.3	40.3	37.2	6.7
96.1 to 100	108	1808	19.8	22.3	24.2	14.3

Percentage classes of diseased crowns	Diseased:			Loss of roots	Average severity on:		
	Crowns	Culms	Roots		Crowns	Culms	Roots
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0.0	0.0	0.0	2.4	1.9	0.0	0.0	10.9
0.1 to 50	36.7	10.7	26.7	12.3	23.3	15.3	37.3
50.1 to 85	63.6	13.0	44.5	27.1	33.8	17.1	43.4
85.1 to 96	89.7	25.0	69.7	31.0	49.5	24.3	54.3
96.1 to 100	99.4	26.6	65.4	42.1	61.9	30.2	66.7

The severity of lesions is expressed as the percentage of the greatest severity possible. The accuracy of this depends on the experience and judgment of the observer. In general it is based on the extent of the lesion and the intensity of the color. A comparison of the roots on a healthy plant with those on a diseased one is the basis for estimating the percentage of roots lost.

The other method of summarizing the data gives either (1) a comparison of the relative severity of the take-all on the crowns, culms, and roots, one group with another when all are diseased (Fig. 1), or (2) records occurrence only when one or two of the above organs or groups of organs are diseased and the third is not diseased (Fig. 2).

DISCUSSION

All the data indicate there is a definite interrelation between both the occurrence and severity of take-all lesions on the crown, culm, and roots. If the crown was not diseased, in only a relatively few cases were the culm and roots diseased. However, there were not many cases in which the roots alone were diseased, indicating that crown infection follows very soon after root infection. During the 5 years' study, only 4 plants were found in which the crown alone was diseased. In these particular instances there may have been diseased roots that were lost in handling.

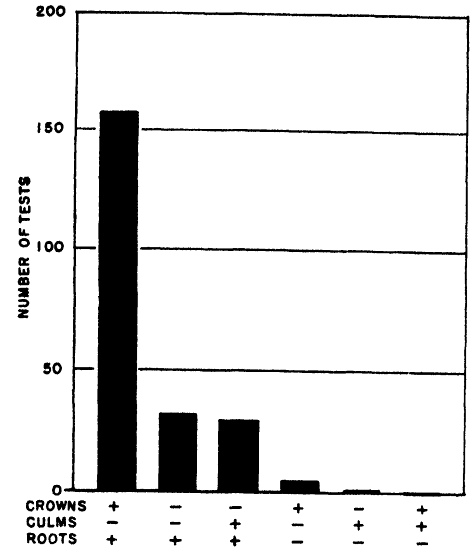

FIG. 1. Coincidence of presence and absence of take-all lesions on crowns, culms, and roots of Kanred wheat plants grown in the greenhouse in naturally infested soil. + indicates presence of lesions; −, absence of lesions.

An increase in the number of plants having diseased crowns is accompanied by an increase in the number of other diseased organs as well as an increase in the severity of disease on all parts. There is a slight exception

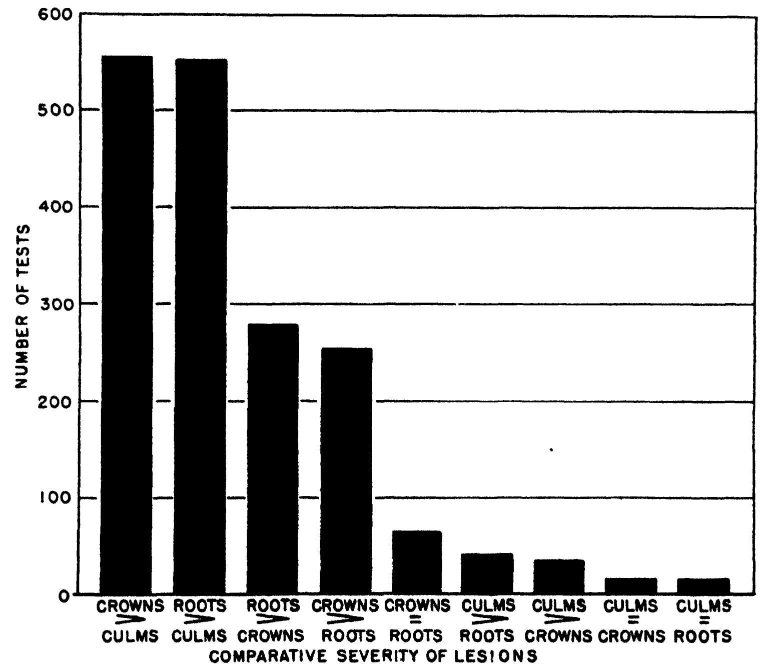

FIG. 2. Interrelations of severity of take-all on pairs of such organs as crowns, culms, and roots of Kanred wheat plants when all three of these organs were diseased. The symbol > indicates "greater than"; =, equal.

in the case of the percentage of roots diseased. This may be explained by the fact that new roots are formed during the life of the plant. Some of these roots may not have had time to become infected, and if infected, the time may have been too short for maximum severity. The loss of roots and the percentage of dead plants also increase with an increasing number of diseased crowns. The average height, the percentage of culms heading, and the percentage of plants tillering decrease, in general, with an increase in the number of crowns diseased. There is one slight exception in height, as shown in table 1.

As shown in table 1, as well as in figure 2, when only one of the 3 groups of organs is affected, it is usually the roots, indicating that they are the first to become diseased. Only once in 5 years has the culm alone been diseased and only 4 times were the crowns alone found to be infected. It is possible in these instances that diseased roots had been present but had been broken off in handling.

When 2 groups of organs were diseased and the third was not, it was found in the great majority of tests that it was the crowns and roots that were diseased and the culms not. The combination of culms and crowns diseased, and the roots free of lesions, has never been found. There were 29 of a total of 157 tests in which the culms and roots were infected and the crowns not (Fig. 1).

A comparison of the severity of lesions on the 3 organs under consideration shows, in general, the lesions of the crown and roots to be the most severe. Instances where the crown severity is greater than the root, and that of the root greater than that of the crown are about equal (Fig. 2).

There were 391 pots in which dead plants were found. In 387 of these, the crown, culm, and roots were diseased. Evidently, a wheat plant must be thoroughly invaded with *Ophiobolus graminis* before death occurs.

Sporulation was observed in 147 tests involving 1522 plants. In every instance where sporulation was noticed, all of the 3 organs under consideration were diseased and 29.7 per cent of the plants were dead. Sporulation was recorded only when it was clearly obvious. Evidently, abundant sporulation is associated with a high percentage of severity of the disease.

The casual observer depends on the black culm lesion for a diagnosis of take-all. If the disease has advanced enough for the typical culm lesions to show, one can be sure that in nearly all cases the crown and roots are diseased, and rather severely diseased.

SUMMARY

Observations were made on the interrelation of different degrees of severity of lesions on the roots, crowns, and culms of wheat plants attacked by *Ophiobolus graminis*. The data presented summarize 5 years' greenhouse work.

A definite interrelation was found between the presence and severity of lesions on the organs under consideration. If one organ alone was diseased,

it was usually the roots. Root infection was followed quickly by crown infection.

Increases in the percentages of crown infection were accompanied (1) by increases in root and culm infections; in the severity of lesions on all parts; in the loss of roots; and in the percentage of dead plants, and (2) by decreases in the average height of plants; in the percentage of culms heading; and in the percentage of plants tillering. All plants killed by *Ophiobolus graminis* and those on which sporulation was observed were found to have roots, crowns, and culms infected.

HISTOLOGY OF PHYMATOTRICHUM ROOT ROT OF FIELD-GROWN COTTON[1]

G. M. WATKINS

(Accepted for publication Nov. 30, 1937)

The mode of entry of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, into the root tissues of susceptible plants has been studied by Peltier and Samson,[2] who reported that the fungus may enter the roots of field-grown cotton and alfalfa through such openings in the periderm as lenticels and wounds, and through ruptures caused by the emergence of lateral roots, or it may actually penetrate the intact periderm by mechanical action. According to them, the large central hypha of the superficial plectenchymatous strand proliferates in the direction of the underlying host cells. The branch hyphae thus formed grow through the external hyphal layers of the strand and produce a mycelial mass on the outside of the periderm. This mass is reported to assume a wedge-like form and to insert itself by mechanical force between the layers of cork cells. More recently, Peltier,[3] in discussing the relation between the strands and infection, has stated that "they are the sole agents responsible for the direct penetration and infection of the roots of susceptible plants." Butler,[4] working with watermelon plants infected with *P. omnivorum*, both in the field and under pure culture conditions *in vitro*, has reported in confirmation of Peltier and Samson that single or grouped hyphae invade the host tissue by a process of mechanical wedging.

In another paper[5] the writer has reported the results of a cytological study of cotton seedling roots infected *in vitro* under pure-culture conditions. This

[1] Published with the approval of the Director as contribution No. 424, Technical Series, of the Texas Agricultural Experiment Station. The progress of this study was greatly facilitated by a grant from the Texas Academy of Science for the purchase of materials and equipment.

[2] Peltier, G. L., C. J. King, and R. W. Samson. Ozonium root rot. U. S. Dept. Agr. Bull. 1417. 1926. (See Part II. The pathological anatomy of Ozonium root rot.)

[3] Peltier, G. L. Distribution and prevalence of Ozonium root rot in the Shelter-belt Zone of Texas. Phytopath. 27: 145–158. 1937.

[4] Butler, K. D. The cotton root rot fungus, *Phymatotrichum omnivorum*, parasitic on the watermelon, *Citrullus vulgaris*. Phytopath. 25: 559–577. 1935.

[5] Watkins, G. M. Cytology of Phymatotrichum root rot of cotton seedlings grown in pure culture. Amer. Jour. Bot. 25: 118–124. 1938.

study showed that infection is caused by newly formed hyphae that grow out from the inoculum, and that the typical buff-colored hyphae and strands do not appear until the attack is well advanced. Sections of the infected seedling roots usually showed degenerative changes in the nuclei and cytoplasm, abnormal shrinkage of protoplasts, and alteration in thickness and staining reaction of walls of cells several layers distant from the nearest invading hyphae. These abnormalities, together with the frequent occurrence of fragments of host cell walls in the midst of hyphal masses in more advanced infections, were considered as indirect but strong evidence of the liberation by growing hyphal tips of chemical substances, perhaps in the nature of enzymes, which facilitate disintegration of root cells in advance of penetration. Since the evidence appeared so convincing in favor of the power of the fungus to break down parenchymatous tissue by means of exudates absorbed in advance of hyphal entry, it has seemed well worth while to reconsider the problem of the histology of root rot of field-grown cotton and to investigate the possibility of similar processes being involved in fungous invasion of the suberized cells, which constitute the outer protective periderm.

The literature relevant to the general problem of host-parasite relationships in fungous diseases of plants has been well discussed by Brown,[6] and mention has been made previously[7] of a number of papers which seem to concern these relationships in cotton root rot.

MATERIALS AND METHODS

The cotton roots used in this study were collected from July to October of 1936 and 1937 from 3 fields in central Texas known to have been planted in cotton for several years and to have exhibited regularly, for a number of seasons, severe losses from typical Phymatotrichum root rot. Two of these places, the experimental root-rot plots of the Texas Agricultural Experiment Station and a commercial cotton farm 3 miles west of Bryan, are in Brazos County. The third is a cotton farm in Travis County. In collecting material for microscopic examination the plants were removed from the soil and the roots inspected immediately for the occurrence of typical hyphae or strands of *P. omnivorum*. Characteristically infected roots were obtained in all stages, from apparently normal roots bearing traces of delicate white hyphae to thoroughly rotted specimens completely surrounded by a buff-colored weft of strands and hyphae. All roots were placed in humid containers, brought directly to the laboratory, and sectioned at once by means of a sliding microtome. The sections resulting from this procedure ranged from 15 μ to 25 μ in thickness and were transferred as fast as cut to a preserving solution. Some of the sections were placed immediately in lactophenol for 15 minutes or more, transferred to another vessel of the same solution to which had been added cotton blue (0.5 gm. per 100 cc.) for 5 to 15 minutes, then

[6] Brown, W. The physiology of host-parasite relations. Bot. Rev. 2: 236–281. 1936.

[7] See footnote 5.

washed by passing through clear lactophenol, and finally mounted on slides in the same solution. Another lot was placed in Flemming's weak solution for several hours, washed in water, bleached in hydrogen peroxide, stained with safranin and light green, and mounted in balsam. The sections stained with cotton blue in lactophenol were better for studying the successive stages in hyphal invasion of the periderm, phloem, and cambium, while the permanent mounts stained with safranin and light green were superior for demonstrating hyphae in the xylem.

The examination of preparations obtained by the above methods has yielded information on the successive stages in the process of hyphal invasion of roots of normal field-grown cotton plants. Typical examples of the most significant stages have been drawn with the aid of a camera lucida.

OBSERVATIONS

The periderm of normal disease-free cotton roots of sufficient size to show advanced development of secondary tissues consists of an outer covering of cork cells, ordinarily 10 or 12 layers thick, with the cork cambium beneath and the phelloderm of parenchyma-like cells produced by the cork cambium on its inner side. As successive layers of cork cells are produced, the outermost layers of this tissue are constantly being sloughed off and lost. From the exterior of the intact cotton root this is evidenced by the appearance of minute cracks in the surface and the rolling back of extremely thin, scale-like layers of cells on both sides away from the original line of breaking. Frequently in roots in the earliest stages of mycelial attack the small white, more or less fluffy wefts are observed growing in the superficial crevices of the outer cork layers.

In transverse sections the ruptures in the surface of the cork tissue are seen to involve usually from 1 to 5 layers of cells, and it is in these crevices that the first advancing hyphae usually become established. At first the hyphae are loosely interwoven, but further growth generally transforms them into a more or less compact cellular mass (Fig. 1, a). As the separated layers of cork cells roll back away from the original rupture, the fungus is able to expand laterally from the original trench that it occupied and not infrequently is observed to extend the length of several cells down into the pocket formed beneath the separating roll of cork cells. The hyphae grow closely against the exposed walls of the cork cells (Fig. 1, a), but have not been observed by the writer to crush or give any indication of having exerted pressure upon the host cell walls.

Perhaps the most frequently observed phenomenon in this material is the change that occurs in the appearance and structure of those cork cell walls against which are lying hyphae of *P. omnivorum* (Fig. 1, a, b, c, d, f). Normally, almost hyaline in unstained mounts, these walls become brown, swollen, and sometimes distorted when the mycelium has grown in close proximity to them for a time. Occasionally, the walls appear under these conditions to be separated into laminae, although perhaps not to the extent frequently ob-

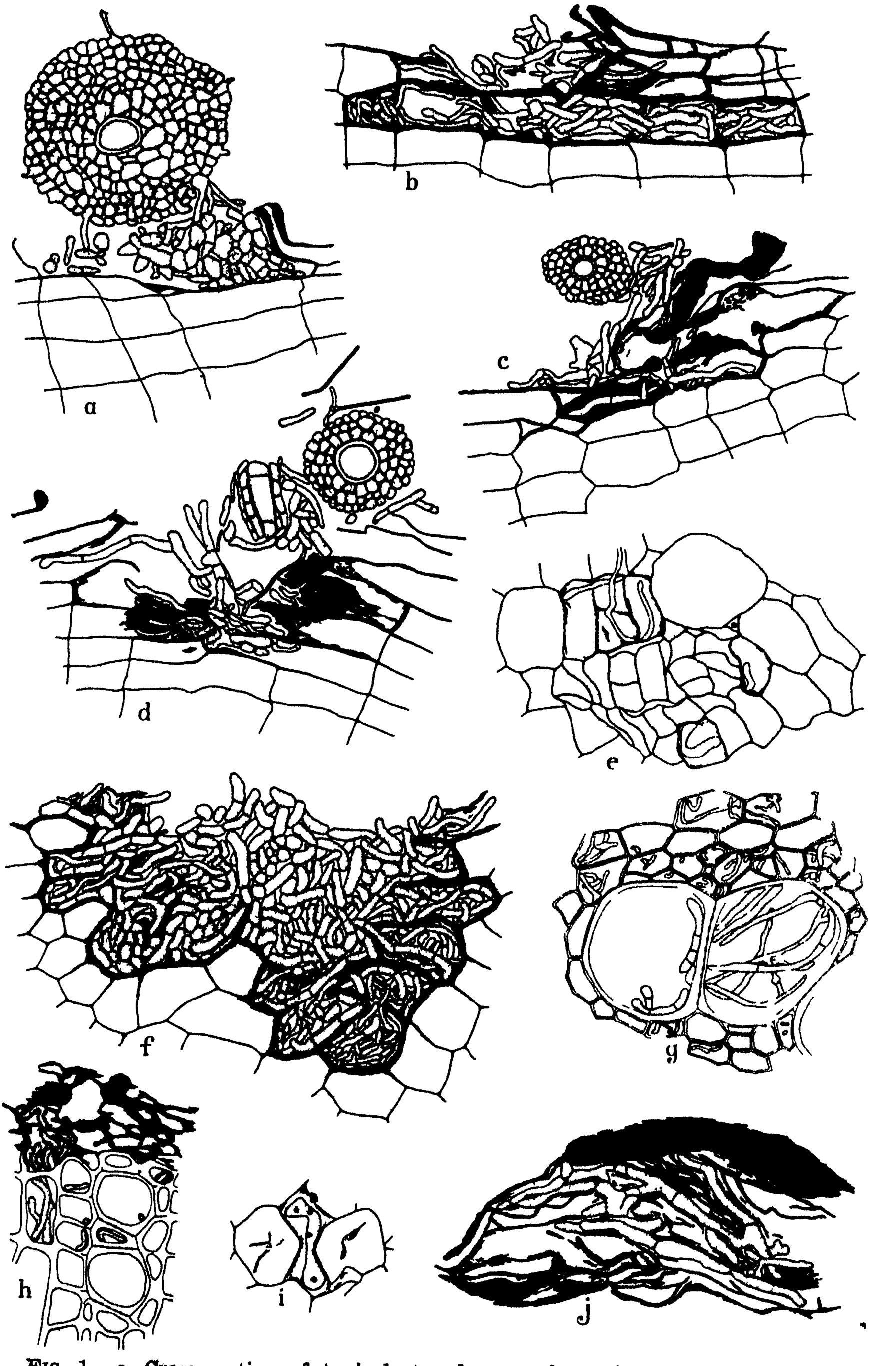

FIG. 1. a. Cross section of typical strand on surface of periderm. A group of hyphae are growing into the pocket at right formed by a separating layer of cork cells. b. Hyphae in a layer of cork cells beneath the surface. All walls in contact with hyphae are swollen and some are distorted. c. Cross section of strand at surface of root. Hyphae breaking down outer cork cells. d. Sections (transverse and oblique) of strands.

served in walls of the cortical parenchyma in seedling roots.[8] After this apparent chemical action on the wall has continued long enough to bring about its structural decomposition, breaks may occur that expose the cavity of the next adjoining cell and one or more hyphae from the accumulated weft soon grow into it (Fig. 1, b, c, d). The process is then repeated and continues in the same manner in all directions from the point of original establishment of the fungus. The spread of the hyphae is often at first more rapid tangentially than radially to the interior. Inasmuch as the hyphae, after entering each newly exposed cell cavity, soon undergo further growth and branching to occupy the additional acquired space, the entire cavity of the lesion comes to be filled with a more or less compact mass of hyphae that resembles a pseudoparenchyma in structure (Fig. 1, b, f). Around the entire zone of contact between this mass and the cork cells of the periderm are seen the remains of cork cell walls engulfed by the advancing hyphal mass but not yet completely broken down (Fig. 1, f). All intact host-cell walls in immediate contact with the hyphal mass show the brownish discoloration and increased thickness characteristic of the walls described above in similar juxtaposition to hyphae in the first stages of invasion. This process of progressive breaking down of successive layers of cork cells, followed promptly by invasion and consolidation of newly opened cell cavities, continues until the fungus has completely penetrated the cork covering of the root.

The further growth of the hyphae through the phellogen, phelloderm, and phloem is apparently a more rapid process. In these tissues products of the growing hyphae appear to be transferred readily to cells several layers distant, for the influence of some toxic substance can often be seen in the degeneration of cells before hyphal penetration occurs. The cells of the phelloderm and many of the older layers derived from the phloem are normally filled with starch grains, which begin to disappear as soon as hyphae enter these tissues. The hyphae proceed between and through the cells, and the invaded tissue soon disintegrates to such an extent that only darkened masses of partially broken-down cell walls and débris from degenerating protoplasts remain (Fig. 1, h, j). Here and there in the remnants of phloem tissue the network of hyphae can be seen (Fig. 1, j). The cells of the cambium and immediately adjacent phloem regions are among the first to collapse after the mycelium has penetrated within the periderm (Fig. 1, h), a condition that makes the observation of hyphae in close connection with cambium cells an infrequent occurrence (Fig. 1, e, i). In the phloem region the numerous

[8] See footnote 5.

Hyphae penetrating into cork cells beneath the surface and causing disintegration of walls. e. Hyphae in cells of cambium (oriented vertically in this illustration) and phloem. f. Hyphal mass that has penetrated about halfway through the periderm. Remains of cork walls are shown among the hyphae around the margin of the lesion. g. Hyphae in vessels, tracheids, and wood parenchyma. Two examples are shown of the passage of hyphae through pits. h. Hyphae in xylem cells and in the disorganized remains of cambium and phloem. i. A hypha between two cells of the phloem. j. Hyphal network in the disorganized phloem tissue. All illustrations × 175, except e and i, which are × 431.

bundles of bast fibers are especially conspicuous after the breakdown of the surrounding cellulose-walled cells. The fibers appear to be extremely resistant to the action of the fungus; and, although in advanced infections hyphae may occasionally be encountered within the lumina of fibers, no case of actual disintegration of bundles of bast fibers has been observed.

Sections of roots in advanced stages of decay usually show hyphae in great abundance in the xylem (Fig. 1, g, h), as noted by Peltier, King, and Samson.[9] At times cross sections of such material show hyphal distribution in more or less distinctly circumscribed areas in the wood, as though the principal direction of spread were lengthwise in the root rather than transverse. This type of hyphal distribution in the xylem may be considered as lending support to the conclusion of Peltier, King, and Samson that the mycelium travels readily in a longitudinal direction within the woody tissues. Radial growth seems to be accomplished more easily through the xylem rays, the cells of which are generally elongated in the radial direction. The slender hyphae so frequently found in the rays may travel more or less directly through several cells in tandem without being greatly diverted from the radial line of growth. The mode of wall penetration here is characteristically through the pits, as is true also of hyphae passing through walls of vessels, tracheids, and xylem parenchyma (Fig. 1, g). There is generally a considerable reduction in the diameter of the hypha at the point of passage. No evidence has been found of any chemical action upon the walls of xylem constituents; even those woody tissues permeated by a dense network of mycelium show no indication of decomposition of walls.

As far as has been observed in this study, the cotton root is unable to produce any defensive mechanism for inhibiting the fungus in its further spread after it has once traversed the periderm. The living cells of the phellogen, phelloderm, phloem, and cambium seem to be readily susceptible to damage considerably in advance of actual hyphal penetration. This, presumably, is brought about chemically by exudates from the active hyphal tips.

The observation of Peltier and Samson that the mycelium of *P. omnivorum* enters the root through lenticels and through ruptures in the periderm caused by the emergence of lateral roots is confirmed in the present work. Hyphae have been seen frequently to accumulate over the outer surfaces of lenticels and to grow through and between the loosely arranged cells beneath. In advanced stages of hyphal entry through lenticels the remnants of partly destroyed host cells are to be observed usually within the invading mass of hyphae, and around the borders of lenticels the mycelium often begins to grow into the exposed cells of the upturned phellem in much the manner described above for hyphal growth into cells beneath the edges of cork layers being sloughed off.

DISCUSSION

In its attack upon the uninjured, uninterrupted portions of the periderm of the cotton root, *Phymatotrichum omnivorum* shows much evidence in cyto-

[9] See footnote 2.

logical preparations of utilizing chemical exudates, probably enzymic in character, as a means of destroying the homogeneity of the matrix of the suberized walls. The process appears to be fundamentally similar to that described elsewhere[10] for the invasion of cotton seedling roots by the same fungus. The chief differences, readily discernible in a microscopic examination, are that, in the case of the attack on the cork cells of the periderm, the process of invasion proceeds much more slowly, and that the apparent changes in the composition and structure of the cork walls are not observed to have taken place farther in the host tissue than the walls immediately exposed to the action of the growing hyphae. In other words there is little apparent action in advance of actual hyphal penetration. This is possibly to be explained on the basis of the impermeability of the suberized walls of the outer periderm to the passage of solutions and the inability of fungous exudates to be absorbed and transferred beyond the intact wall next adjacent to the growing hyphae. The necessarily slow progress of the fungus through the resistant periderm accounts for the absence of the individual hyphal penetration through cell after cell so frequently seen in parenchymatous tissue. Since progress is slow and each cork wall is broken down only after being subjected to the action of the accumulated products of many hyphae, the fungus has opportunity for complete occupation of each cell cavity before the next one is opened.

In this work no examples have been encountered of the crushing of periderm cells adjacent to an advancing hyphal mass, and it thus seems unlikely that the penetration of the mycelium is due solely to mechanical force. On the other hand there is evidence strongly suggestive of the power of the actively growing hyphal tips to secrete and liberate some substance or substances that have the property of softening and dissolving suberized cell walls.

Present observations emphasize the dominant rôle in infection played by the actively growing portions of the mycelium. The fact that, in more than half the infection sites studied, the invading hyphae were not immediately associated with superficial plectenchymatous strands, tends to discount the necessarily direct relation between strand and invasion supposed by Peltier and Samson.

SUMMARY

In naturally occurring root rot of field-grown cotton the fungus, *Phymatotrichum omnivorum*, forms hyphal wefts that grow over the surface of the periderm and frequently accumulate in the superficial crevices resulting from the rupture and sloughing-off of the external cork layers. The periderm cell walls in immediate contact with hyphal agglomerations soon begin to show changes in structure, color, and thickness that suggest chemical action upon them by substances possibly liberated from the actively growing hyphae. Breaks occur in such walls, permitting the fungus to invade the newly

[10] See footnote 5.

opened cell cavity. As this process continues the number of invaded cells increases, and the fungus thus progresses slowly through the periderm. The center of the lesion is occupied by a compact hyphal mass that contains in its interior the engulfed remnants of partly destroyed cork walls. After having penetrated the phellem, the mycelium spreads rapidly through the phellogen, phelloderm, phloem, and cambium, causing widespread collapse and disintegration of cells, and enters the woody cylinder. The passage of hyphae from cell to cell of the xylem is chiefly through pits, since the lignified walls of these cells are not readily broken down by the fungus.

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PECTASE ACTIVITY OF CERTAIN MICROORGANISMS[1]

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Pectic enzymes are known to occur generally in tissues of higher plants and within cells or in secretions from certain microorganisms. In certain plant diseases characterized by the dissolution of cells in advance of the invading pathogen, the pectic cementing materials binding the structural elements of plant tissues are broken down, presumably by the pectic enzymes secreted by the pathogen. These enzymes include protopectinase, pectase, and pectinase, according to the nomenclature suggested by Davison and Willaman (3). Pectic activity of microorganisms having a relationship to parasitism has been determined by methods based upon the dissolution of cell walls (by protopectinase) of selected tissues (2), (6), and (8), the time of coagulation and consistency (by pectase) of pectic gels (3), and end products (by pectinase) from pectin (3). Certain disadvantages encountered with these methods and the importance of pectic enzymes in diseases of plants prompted the investigations on a method (10) for determining pectase activity based upon the hydrolysis of a known substrate. Pectase hydrolysis disrupts the methyl ester linkage in pectin with the production of methyl alcohol and pectic acid which, in the presence of calcium ions, goes to calcium pectate. According to Neuberg and Ostendorf (10) extracts from plant tissues possessing pectase activity hydrolyze the ester linkage of half calcium salt of monomethyl tartaric acid. Since the substrate is water-soluble and is hydrolyzed by pectase into soluble methyl alcohol and insoluble half calcium salt of tartaric acid, the method has promise of quantitative measurements based upon the precipitate formed. The purpose of this paper is to call attention to this method and to report the data obtained from determining pectase activity of certain plant pathogens, available in the laboratory, and an extract of cured-tobacco leaves by this method.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.
[2] Now with U. S. D. A. Bureau of Plant Industry, Washington, D. C.

METHODS

The various organisms were cultured for 5 days at 37° C. in 100 mls. of nutrient broth containing 10 per cent commercial pectin, which was added to favor the production of pectase (7) and (8). At the end of the incubation period the cultures were adjusted to a reaction of pH 8.5 in 0.1 molar phosphate buffer and filtered through filter paper. The mycelial mass of the fungal pathogens in the residue and 25 grams of cured-tobacco leaves were macerated separately with mortar and pestle and then extracted in 100 mls. of 0.1 molar phosphate buffer at pH 8.5 for 6 hours at room temperature. The filtrates from these extractions and the filtrate from the culture fluids were added to an equal volume of 95 per cent ethyl alcohol for precipitation of the enzymes (1). The precipitated materials were collected on filter paper and, after being dried rapidly, were kept until needed in the experiments. The dried material was extracted in 2 mls. of 0.1 molar phosphate buffer at pH 8.5 and the clear liquid after passage through filter paper was used immediately as the preparation of pectase.

The ester substrate (half calcium salt of mono-methyl tartaric acid) was prepared[3] according to the method of Guérin-Varry (5), since the substance was not purchasable at the time. Five mls. of a 10 per cent aqueous solution of the crude syrup of the ester (containing also di-methyl ester) after being adjusted to a reaction of pH 6.5 in acetate buffer was added to the pectase preparations. Upon mixing the liquids and adding 0.5 ml. of toluol to prevent bacterial growth, the solutions were incubated at 37° C. for 1 to 5 days. A precipitate in the tubes indicated hydrolysis, which is considered to be pectase activity.

RESULTS

Qualitative data on the hydrolysis of the ester substrate by the preparations from cultures of the pathogens and from cured-tobacco leaves are given in table 1. They indicate hydrolytic activity from certain cultures and from tobacco tissues. The cultures of bacteria possessed but slight activity. Those of *Fusarium* that had been transferred several times on artificial media since isolation from diseased tobacco stems showed very slight or no activity, while freshly isolated cultures gave moderate hydrolysis. The cultures of *Sclerotium* gave good tests, while those of *Sclerotinia* yielded but a fair amount of precipitate. Two specimens of a culture of *Rhizoctonia* that had been isolated for some time from diseased tobacco stems gave negative tests. The cultures of *Thielaviopsis* yielded positive but varying results. Upon artificial inoculation[4] culture 538, which possessed moderate pectase activity, produced severe black-root rot of tobacco, while culture 535, with slight pectase activity, produced less severe rotting. Culture 536, with marked pectase activity, was not inoculated. Extracts from cured-tobacco leaves possessed a fair amount of activity.

[3] Aided by Dr. Charles Barkenbus, Department of Chemistry, University of Kentucky.
[4] Unpublished data furnished by Dr. E. M. Johnson.

TABLE 1.—*Qualitative determinations of pectase activity of various plant pathogens and cured-tobacco tissue*

Organism		Material	Results
Phytomonas mori (B. & L.) C. S. A. B.		Fluid	+[a] slight
P. tabaca (W. & F.) C. S. A. B.		"	+ "
P. angulata (F. & M.) C. S. A. B.		"	+ "
Bacteria from tobacco stem		"	+ "
Fusarium sp. from tobacco stem	484	"	++
F. " " " "	439	"	–[b]
F. " " " "	436	"	+ slight
F. " " " "	434	"	–
F. " " " "	477	"	+ slight
F. " " " "	302	"	+ "
F. " " " "	255	"	+ "
F. " " " "	227	"	+ "
F. " " " "	(freshly isolated)	"	++
F. " " " "	(all above combined)	Mycelia	+
Sclerotium bataticola Taub.	1	Fluid	+++
S. "	2	"	++
S. "	(1 and 2)	Mycelia	++
Sclerotinia sclerotiorum (L.) Mass.		Fluid	+
S. trifoliorum Erik.		"	+
Rhizoctonia sp. from tobacco	540	"	–
Thielaviopsis basicola (B. & B.) Zopf	535	"	+ slight
T. "	538	"	++
T. "	536	"	+++
T. "	(all above combined)	Mycelia	+++
Tobacco (tissue extract)		Fluid	+
Control (without enzyme)			–

[a] Amount of precipitate, positive test.
[b] Absence of precipitate, negative test.

DISCUSSION

The tartaric acid-ester method of measuring pectase hydrolysis appears to be adaptable for studies on plant pathogens, since positive results were obtained from certain organisms in culture. Because a known substrate is used this method may be more useful than those methods previously employed. Since plant tissues may contain inactive pectic enzymes capable of being activated by treatment or by definite substances, the tissue method might lead to erroneous results if the preparation tested for enzyme activity contained activators. Under such conditions enzymes present in the tissue would be measured instead of, or along with, that of the preparation. Davison and Willaman (3), by their method of preparing pectin, eliminated enzymes from the substrate, but their method of measuring enzyme activity requires considerable time and apparatus. The tartaric acid ester method (10) eliminates enzymes from the substrate and affords a simple and rapid means of determining pectase activity. It is adaptable for quantitative measurements.

Since the characteristic nucleus of the pectic materials appears to be digalacturonic acid (9), pectase presumedly acts on the ester linkage of the acid with methyl alcohol in the pectin complex. Some evidence (10) indicates that lipase from *Ricinus* seeds also hydrolyzes the methyl ester linkage in

pectin. Since pectic enzymes are generally present in plant tissues this lipase may have contained pectase.

Alteration of the physical and chemical properties of soluble pectin by the action of pectase may account for certain pathological expressions, although the rôle of pectase in the symptoms of plant diseases is not known. The substrate for other pectic enzymes (protopectinase and pectinase) is not definitely known, but the enzymes are apparently associated with certain pathogens. In plant diseases characterized by dissolution of the cells in advance of the pathogen, protopectinase (in some references pectinase) has been considered the causative agent. Its action changes the insoluble pectic material, cementing the structural elements of cell wall into soluble pectin. Since the action of an enzyme on an insoluble substrate is difficult to explain, the dissolution of cells may result from the action of pectase on soluble pectin that may be present in the cell walls. The pectic acid produced from such hydrolysis would be precipitated by the calcium ions in the tissue to insoluble calcium pectate (4), which has gelling properties. The forces of gelation of the hydrophyllic calcium pectate might disrupt the cell walls.

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A SAND-NUTRIENT INFECTION TECHNIQUE FOR THE STUDY OF FUSARIUM WILT OF COTTON

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(Accepted for publication Dec. 1, 1937)

Investigations of the nutritional relations of fusarium wilt of cotton at the Arkansas Agricultural Experiment Station have necessitated the development of a sand-nutrient infection technique. The method reported here was first used in modified form to determine the influence of variations in the nutrient supply on the relative incidence and severity of cotton wilt under greenhouse conditions. Through the information obtained from repeated trials it has been found effective to apply inoculum as a 3-day growth of the pathogen on modified Richard's solution. By growing the organism at 28–30° C. and keeping it well dispersed in the solution by shaking, it was found to produce a maximum of spores and active mycelium fragments with a minimum of staling products in the 3 days. This exclusion of all possible organic material and fungus staling products seemed necessary to the accurate determination of nutrient effects. The method described is relatively rapid, since preparation can be completed within a week and a significant disease differential obtained within 6 weeks after planting. These preliminary experiments showed such promise that the method was tested on a series of cotton varieties of known[1] relative wilt resistance under field conditions. The comparative resistance of these varieties was shown to be relatively the same, by this method of testing in the greenhouse, as has been demonstrated under very severe field conditions.

THE METHOD

Twenty-four 3-gallon glazed earthenware jars, with center drainage holes in the bottom, were equipped with siphon drains so that moisture would be automatically removed if the level of the liquid rose over 1½ inches from the bottom (Fig. 1). These jars were then filled to within two inches of the top with washed river sand that had been pasteurized[2] at 80° C. Three days prior to planting an actively pathogenic isolate of *Fusarium vasinfectum* Atk. was inoculated into 24 1-litre Erlenmeyer flasks, each containing 500 cc. of sterilized Richard's solution made up in tap water with iron omitted. These flasks were shaken vigorously every 8 hours and incubated for the 72 hours at 28°–30° C. One flask of the inoculum was then mixed thoroughly

[1] Ware, J. O., V. H. Young, and G. Janssen. Cotton wilt studies. III. The behavior of certain cotton varieties grown on soil artificially infested with the cotton wilt organism. Arkansas Agr. Expt. Sta. Bull. 269. 1932.

[2] The ½-yd.-capacity pasteurizer used was made according to the directions supplied by the General Electric Company, from which the heating units were purchased. The use of this type of pasteurizer is described by J. G. Horsfall. Pasteurizing soil electrically to control damping-off. New York (Geneva) Agr. Expt. Sta. Bull. 651. 1935, and by Newhall, A. G., and M. W. Nixon. Disinfesting soils by electric pasteurization. New York (Cornell) Agr. Expt. Sta. Bull. 636. 1935.

with the sand in each of the 24 jars. An additional inch of moist, pasteurized river sand was spread over the surface of each jar, then 20 sulphuric acid-delinted seeds were planted in each jar in a circle 1 inch from the periphery and 1 inch deep. Four jars were planted to each of the 6 varieties: Half and Half (Summerour's), Misdel-3, Rowden 2088 (Arkansas), Dixie Triumph-6 (Arkansas), Rhyne's Cook, and Sea Island (Seabrook).

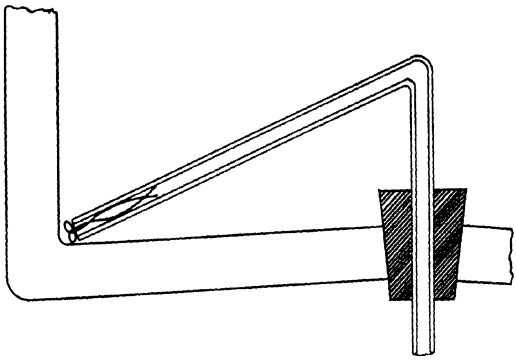

FIG. 1. Siphon drain for sand nutrient jars. The 3 mm. bore glass tube is shaped to have the bend 1½ inches above the bottom of the jar. A fine tinned wire is bent and placed in the upper end of the tube to prevent clogging with sand.

The nutrient solution was added at the rate[3] of 1 quart per jar per day, starting on the day following planting and continuing without flushing until the termination of the experiment. The nutrient formula used for this experiment is given in terms of milliliters of half molar solutions of salts to be made up to 10 quarts with tap water.

Calcium nitrate, 12.5 ml.; ammonium nitrate, 7.25 ml.; potassium acid phosphate, 7.25 ml.; magnesium phosphate (primary), 7.25 ml.; magnesium sulphate, 7.25 ml.; (all chemicals used were of C.P. quality).

The plants were thinned to 10 per jar on the 14th day and then the second inoculation, using inoculum prepared in the exact manner previously described, was applied at the same rate as before. This time, instead of mixing with the sand, it was poured into a circular depression made 1 inch inside the circle of plants by pressing an inverted Büchner funnel, 5 inches in diameter, into the sand to a depth of about 2 inches. The sand was again leveled. Inoculum was prepared in this same way and introduced into the jars in this same manner on the 21st day following planting and again on the 28th.

This experiment was conducted from July 26 to September 18, 1937. For the first 5 weeks of this period, during which time essentially all of the severe wilt occurred, the mean, daily air temperature of the greenhouse averaged 29.18° C. This temperature is rather close to the optimum reported for this disease under greenhouse conditions.[4] Preliminary experiments

[3] Rate and concentration of nutrients used in the preliminary experiments were suggested by Dr. Cecil H. Wadleigh, Assistant Agronomist, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

[4] Young, V. H. Cotton wilt studies. I. The relation of soil temperature to the development of cotton wilt. Arkansas Agr. Expt. Sta. Bull. 226. 1928.

TABLE 1.—*Development of fusarium wilt in 6 varieties of cotton in sand-nutrient culture. Plants grown in 3-gallon jars in the greenhouse from July 26 to September 18, 1937. Average daily mean air temperature of the greenhouse for the first 35 days: 29.18° C.*

Variety	No. of jars	Total plants	Plants with advanced wilt symptoms by—			Final disease analysis (54 days)					
			28 days	35 days	42 days	Plants entirely dead	Plants dwarfed and badly blighted	Plants slightly blighted	Plants internally discolored but having no external symptoms	Plants healthy	Total having external symptoms
			Per cent	*Per cent*	*Per cent*						*Per cent*
Half and Half (Summerour's)	4	40	100	100	100	40					100
Misdel #3	4	40	90	100	100	36	1	3			100
Rowden 2088 (Arkansas)	4	40	72.5	80	80	28	3	7	2		95
Dixie Triumph #6 (Arkansas)	4	40	32.5	55	55	21	5	8	6		85
Rhyne's Cook	4	40	17.5	22.5	25	8	6	9	17		57.5
Sea Island (Seabrook)	4	40	0	0	0				29	11	0

FIG. 2. Fusarium wilt in 6 varieties of cotton grown for 49 days in artificially infested sand nutrient cultures. A. Half and Half (Summerour's). B. Misdel #3. C. Rowden 2088 (Arkansas). D. Dixie Triumph #6 (Arkansas). E. Rhyne's Cook. F. Sea Island (Seabrook).

with this method demonstrated that when temperatures are very much higher or very much lower than the optimum a high incidence of disease failed to develop. The data obtained from this experiment are shown in table 1 and the diseased condition of the different varieties, after 49 days, is shown in figure 2.

The first wilt in any variety (Rowden 2088) was recorded on the 21st day and within 1 week of this time the entire 40 plants of the most susceptible variety (Half and Half) were all showing advanced wilt symptoms. It was thus necessary to take notes at least once each week, for, quite obviously, *time* becomes one of the elements in measuring resistance when such rapid development and such high incidence of disease are induced.

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INOCULATIONS WITH FOREST TREE RUSTS

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In June, 1927, near Woodgate, N. Y., several species of pine and of the Scrophulariaceae were inoculated with the aeciospores of the *Peridermium* known as the Woodgate rust. The inoculations were kept moist by the use of a celluloid "iceless refrigerator," which is a modified form of the inoculation chamber described by Hubert.[1]

The 1927 terminal leaders, side shoots, and side branches of 5- to 20-year-old pines were moistened thoroughly and were smeared with aeciospores by means of a small artist's brush. A celluloid cone, which was 22 inches in length and respectively 3 inches and 1–1½ inches in diameter at the ends, was slipped over the inoculated parts. It was anchored to the stem by means of a copper wire. A strip of absorbent cotton, about 1 inch in width and 8–10 inches in length, which was saturated with water, was then pushed by means of a small rod through the smaller end, well down the inside of the cone. The entire outer surface of the cone was wrapped to a thickness of 1–1½ inches with wet absorbent cotton. The larger end of the cone was left almost entirely open, the smaller almost entirely closed, leaving an opening sufficiently large for circulation of air through the cone. If the cone was on the terminal shoot of the main axis of the tree, it was supported by one or two sticks, wired to the trunk of the tree; if on a side branch, it was supported from the ground by means of a forked stick (Fig. 1). The cones were left on the shoots for 24 to 36 hours. At the end of 36 hours the cotton on the outside of the cone was quite damp and it was always possible to wring water from the strip of cotton inside the cone. The entire outer surfaces of the shoots were also quite damp.

About 200 shoots of *Pinus resinosa, P. sylvestris, P. radiata,* and *P. ponderosa* were inoculated by this method. In all cases, infection spots appeared on the stems within four to six weeks, and in many cases, on *Pinus sylvestris,* the spots were so abundant that the entire inoculated stem surface became reddish brown in color by the latter part of August. This was equally true of trees which had no galls on them at the time the inoculations were made and of those which had galls.

At the time the shoots were inoculated, comparative germination tests of aeciospores from collections of various ages were made in the inoculation chambers and in water cultures in the laboratory. The water cultures were made by dusting the spores on fresh well-water in wine glasses. In the inoculation chambers pieces of thoroughly clean, white China silk about 2 cm. in length and 1 cm. in width were laid on the pine stems and the aeciospores were dusted on them. The silks were examined under the microscope 24 to 36 hours later. The germ tubes were very much branched and

[1] HUBERT, ERNEST E. Celluloid cylinders for inoculation chambers. Phytopath. 6: 447–450. 1916.

anastomosed so much with one another that they formed very complicated mycelia. In the water cultures, the germ tubes of the same age were comparatively unbranched and unanastomosed. In all of these experiments the percentage of germination in the inoculation chambers was far greater than that in the water cultures. For example, the germination of spores which had been kept in Syracuse watch glasses in the laboratory away from direct sunlight for about 10 days was approximately 25 per cent as compared with 75 per cent in the inoculation chambers.

FIG. 1. Celluloid "iceless refrigerator" used in inoculation experiments with the accio spores of the Woodgate rust on *Pinus sylvestris* and other species of hard pines.

It is possible that Woodgate rust may prove to be one of the western gall rusts, whose alternate hosts are species of the Scrophulariaceae. Although several species of Scrophulariaceae occur in the region where the Woodgate rust has been found, none of them has ever been found infected with any rust. During the latter part of June, 1927, *Chelone glabra* and *Scrophularia leporella* were inoculated with aeciospores of the Woodgate rust. These two species were selected because of their size and because they occur nearby.

The plants when inoculated were about 26 inches in height. Both sides of the leaves were thoroughly moistened and smeared with aeciospores as mentioned above for pines. A stake about ¾ inch in diameter, wrapped with strips of wet absorbent cotton to a thickness of 1½ inches was thrust into the ground beside the plants, so that its upper end extended 4 inches

above them. A celluloid cylinder, 22 inches long and 5 inches in diameter, was placed over the plants and stake, and rested on the ground. It was wrapped with wet absorbent cotton, which extended almost to the tip of the stake so as to leave the end of the cylinder partly open. After 48 hours, the cotton on the stake was wet and the leaves were quite moist. Microscopic examinations were made of pieces of the inoculated leaves soon after the cylinders were removed. The aeciospores had germinated as abundantly and the germ tubes were as profusely branched and had fused as freely as described above. No infection resulted in any of these experiments.

The writer and his assistants have made several thousand field inoculations with aciospores, urediniospores, and sporidia of *Cronartium ribicola,* using the celluloid cones, the celluloid cylinders previously mentioned, and the iceless refrigerators of the type described by Hunt[2] and Snell.[3] The results with the celluloid cylinders were almost entirely negative; those with the "Hunt iceless refrigerator" were fairly satisfactory, though less so than anticipated. Considerable labor is involved in using the "Hunt iceless refrigerator" in the field, especially in carrying water. The celluloid "iceless refrigerator" described above is the most convenient, dependable and labor-saving device which the writer has used thus far for field inoculations with forest tree rusts.

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THE INFLUENCE OF MINERAL NUTRITION ON THE REACTION OF SWEET-CORN SEEDLINGS TO PHYTOMONAS STEWARTI

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(Accepted for publication Nov. 3, 1937)

Several workers (1, 2, 6, 7, 8, 10) have observed that the reaction of certain plants to infectious agents may be altered by varying the mineral composition of the nutrient solution. In connection with experiments being conducted in this laboratory with bacterial wilt of maize, the causal agent of which is *Phytomonas stewarti* Bergey *et al.* (*Bacterium stewarti* E. F. S.), it seemed desirable to ascertain whether the host-parasite relationship of this disease could be modified by mineral nutrition. A study was undertaken, therefore, to determine the influence of nitrogen, phosphorus, and potassium nutrition on the reaction of sweet-corn seedlings to *P. stewarti.* The responses shown by the seedlings are reported in this paper.

MATERIALS AND METHODS

Sweet-corn seedlings of the variety Golden Bantam were grown in white quartz sand previously washed with tap water. Five seeds were placed in each 4-inch porous clay pot, covered with a layer of sand about one-half inch in depth, and kept moist with tap water. When the seedlings had reached a height of 3 or 4 cm., usually 8 days after planting, they were thinned to 3 seedlings in each pot. Twenty-five pots were started for each nutrient treatment, but at time of inoculation 5 pots in each treatment were eliminated. The pots, set in saucers, were so placed on a greenhouse bench that all were exposed to approximately the same environmental conditions.

Nutrient treatments were started 7 or 8 days after planting. Each pot received 100 cc. of nutrient solution 3 times a week. The composition of the solutions is given in table 1. The salt proportions in these solutions

TABLE 1.—*Composition of nutrient solutions used in the different nitrogen, phosphorus, and potassium experiments*

Stock solutions (0.5 molar)	Volume of stock solutions per liter of nutrient solution in experiments on		
	Nitrogen	Phosphorus	Potassium
	cc.	*cc.*	*cc.*
KH_2PO_4	6.3		
$MgSO_4$	7.1	7.1	7.1
$Ca(NO_3)_2$		2.9	2.9
$CaCl_2$	2.9		
$(NH_4)_2SO_4$		2.8	
$NH_4H_2PO_4$			5.6
NH_4NO_3	0–142.9		
NaH_2PO_4		0–161.3	0.7
K_2SO_4		3.2	0–76.7

were based on solution $T_3R_2C_2$ of the Jones-Shive series (3). This solution was chosen because it had produced good growth of corn in preliminary tests. In addition to the salts listed in table 1, boron as H_3BO_3 and manganese as $MnSO_4 \cdot 2H_2O$ were added to all solutions in concentrations equivalent to 0.5 p.p.m. of each element. Sufficient $FeSO_4$ was added to prevent chlorosis from iron deficiency. No attempt was made to hold the osmotic concentration of the solutions constant. The concentrations of nitrogen, phosphorus, and potassium were varied in separate experiments. When the concentration of one of these elements was varied, the concentrations of the other two were held constant.

The seedlings were inoculated 2 or 3 days after the first application of nutrients. A virulent, single-colony isolate (B–1011), described by McNew (5), was used as the stock culture. The culture was maintained on nutrient-dextrose agar slants at 20° C. and subinoculated into 200 cc. of nutrient broth (Difco), which had previously been supplemented with 0.5 per cent dextrose and adjusted to a pH of 6.8 to 7.0. After incubating for at least 72 hours, the culture was injected into the crown and leaf whorl of 30 seedlings in each group. The 30 seedlings remaining in each group were similarly injected with the sterile filtrate obtained by twice filtering the culture through Berkefeld "W" cylinders. These injections subjected all seedlings to the same inoculation wounding and to such metabolic by-products of the bacterium as might be present in the culture.

About 10 days after inoculation the total number of leaves, the number of killed leaves, and the number of necrotic lesions on each plant were recorded. The severity of infection was expressed as an infection index, based upon the average number of necrotic lesions per leaf. Since some leaves collapsed from a diffuse wilt or a coalescence of lesions, an accurate count of the number of necrotic lesions on these collapsed leaves was impossible. Each collapsed leaf, therefore, was arbitrarily classified as having 3 lesions. This calculated number of lesions on the collapsed leaves was added to the number of lesions actually recorded and the sum divided by the total number of leaves. The quotient so obtained was designated as the infection index.

After 18 or 20 days of nutrient treatment, the green weights of leaves and stems were determined. Dry weights were determined after the samples had been dried to constant weight at 100° C. in an electric oven.

Each nitrogen, phosphorus, and potassium experiment was carried out independently, since sufficient greenhouse space was not available to conduct more than one at a time. Therefore, the results of one experiment can not be directly compared with those of another, because of possible changes due to environmental factors. However, the experiments were all carried out in the same manner and for approximately the same duration of time, the nitrogen and phosphorus experiments in November and the potassium experiment in the preceding September. Each experiment was repeated at least once, with compatible results.

EXPERIMENTAL RESULTS

Influence of Nitrogen Supply on Host Reaction

The influence of variations in the nitrogen supply on the reaction of corn seedlings to *Phytomonas stewarti* was studied in the first experiment. The basic nutrient solution is described in table 1. Nitrogen was added as NH_4NO_3 in amounts ranging from 0 to 200 mg. of nitrogen per 100 cc. of solution. In all treatments the phosphorus concentration was held constant at 12.5 mg. and the potassium concentration at 10.0 mg. per 100 cc. of solution.

From the experimental data recorded in table 2, it is obvious that nitrogen

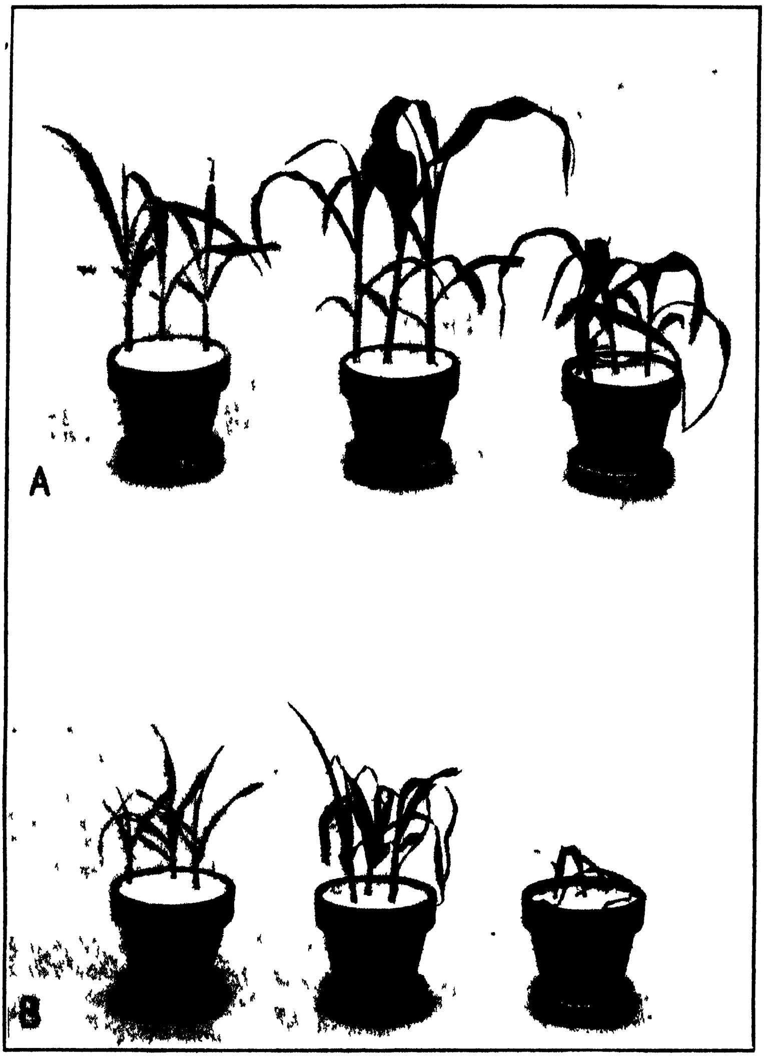
TABLE 2.—*Influence of nitrogen supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Nitrogen added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
				Invaded			
	Healthy	Infected	Total	Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.19	.16	124	29	1	47	.40
10	.40	.28	141	57	1	79	.58
20	.43	.23	119	46	5	63	.66
30	.45	.19	140	61	2	93	.71
40	.42	.25	123	65	5	108	1.00
70	.36	.23	131	58	5	109	.95
100	.32	.17	127	70	6	113	1.03
150	.28	.15	117	62	15	106	1.29
200	.19	.10	109	57	15	92	1.26

supply influenced very markedly the growth of corn seedlings in sand. Relatively low nitrogen levels (20–30 mg.) favored the maximum growth of seedlings. As the nitrogen level was raised above 30 mg., the seedlings made only limited growth. Representative seedlings of 3 different treatments were photographed 10 days after inoculation with the sterile filtrate of the culture. These seedlings, shown in figure 1, A, had received 0, 40, and 200 mg. of nitrogen, respectively, 3 times each week. The seedlings that had received no nitrogen were light yellowish-green and made very little growth. The lower leaves became shriveled and dry. Slight injury, due unquestionably to nitrogen toxicity, was noticeable in seedlings that had received 200 mg. of nitrogen 3 times each week. This injury was apparent as a wilting at the tips of older leaves. Some of these leaves later became entirely flaccid.

The data in table 2 on the reaction of the seedlings to the disease show that infection became more severe as the concentration of nitrogen increased. When more than 150 mg. of nitrogen were added per 100 cc. of solution, severity of infection remained fairly constant. Figure 1, B, shows infected seedlings of the same age and of the same nutrient treatment as those in figure 1, A. In the absence of nitrogen, seedlings showed primary invasion, but little or no secondary invasion. The necrotic lesions produced were small

and few in number. None of the nitrogen-deficient seedlings wilted. As the nitrogen level of the nutrient solution was raised, the pathogen produced larger and more numerous necrotic lesions, and caused a wilted condition of many of the invaded leaves. At the higher nitrogen levels, this type of



Photograph by J. A. Carlile

FIG. 1. Typical corn seedlings that had received 0, 40, and 200 mg. of nitrogen, respectively, 3 times each week, photographed after 2 weeks of nutrient treatment. A. Healthy seedlings. B. Infected seedlings 10 days after inoculation.

wilting was so intense that about one-half of the inoculated seedlings died within 2 weeks after inoculation. Thus, it is evident that infection was more severe at high nitrogen levels than at levels more favorable for plant growth. The possible significance of these results will be discussed later.

Influence of Phosphorus Supply on Host Reaction

In the second experiment, the influence of variations in the phosphorus supply was studied. The phosphorus levels of the solutions ranged from 0 to 250 mg. per 100 cc. of solution. The composition of the basic solution is given in table 1. The required amounts of phosphorus were added as NaH_2PO_4. In all solutions the nitrogen concentration was held constant at 8 mg. and the potassium concentration at 12.5 mg. per 100 cc. of solution.

The experimental data on the relation between phosphorus supply and growth of corn seedlings are given in table 3. These data show that the

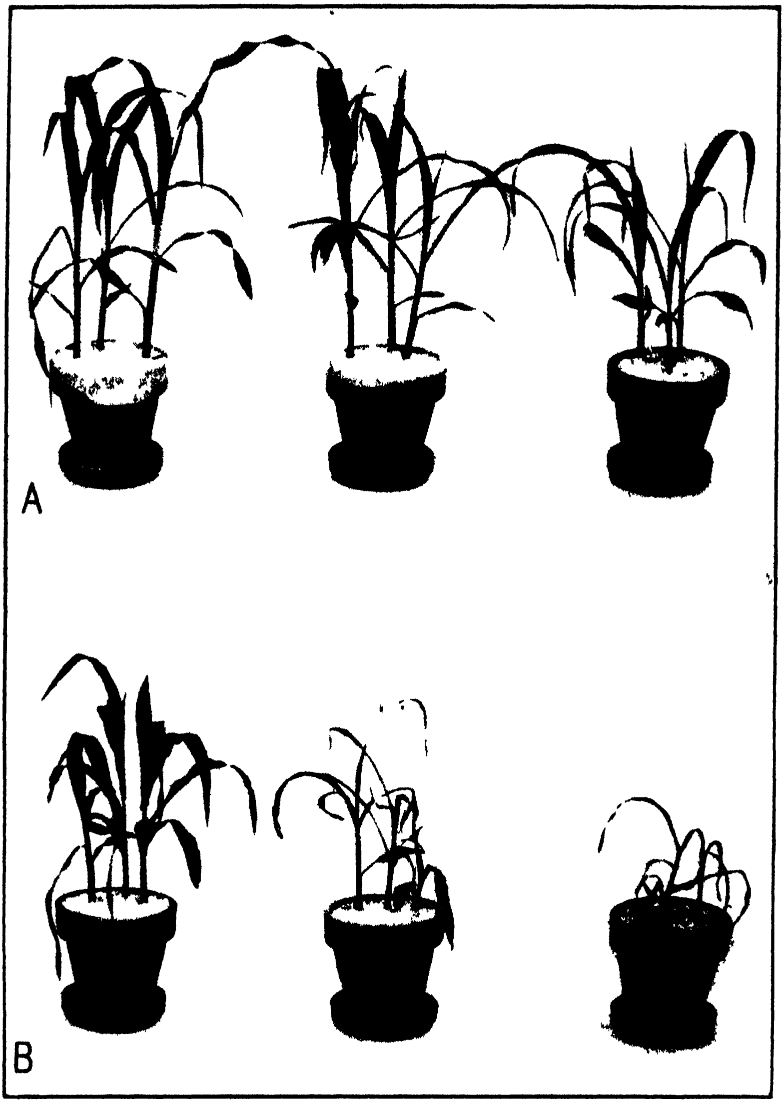
TABLE 3.—*Influence of phosphorus supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Phosphorus added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
				Invaded			
	Healthy	Infected	Total	Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.34	.25	118	60	0	96	.81
20	.41	.25	115	68	2	109	1.00
40	.39	.32	122	73	1	121	1.02
60	.42	.29	121	69	2	125	1.08
80	.35	.28	117	60	3	123	1.13
100	.33	.25	113	66	4	126	1.22
150	.32	.24	113	67	5	116	1.16
200	.28	.19	110	68	4	128	1.27
250	.26	.17	103	63	8	121	1.41

plants grew poorly when more than 60 mg. of phosphorus were added 3 times each week. Maximum growth of seedlings was obtained when 20 to 60 mg. were added 3 times each week. Figure 2, A, shows pots of representative seedlings that had received 0, 100, and 200 mg. of phosphorus, respectively, 3 times each week for 3 weeks. Seedlings were injured somewhat by solutions containing either a deficiency or an excess of phosphorus. The seedlings that had received a solution deficient in phosphorus were purplish, with some yellowing apparent on a few of the lower leaves. These seedlings were slightly smaller than those that had received small amounts of phosphorus. The seedlings that had received the largest amounts of phosphorus grew poorly, but the only noticeable injury was a wilting at the tips of the lower leaves.

Figure 2, B, shows infected seedlings, on the same nutrient treatments as those in figure 2, A. At low phosphorus levels infection was characterized by the formation of definite necrotic lesions. At the higher phosphorus levels infection was manifested not only by necrotic lesions but also by dwarfing of the seedlings and a general wilting of the invaded leaves. This wilting was not intensified to the extent that death resulted within 2 weeks following inoculation, as was the case with the high-nitrogen seedlings. The infection gradually became more severe with increasing amounts of phosphorus, even though these high levels of phosphorus retarded seedling growth.

Since phosphorus was added as NaH_2PO_4, the sodium concentration increased simultaneously with that of phosphorus. In order to determine whether the increased severity of wilt at high levels of NaH_2PO_4 was due to phosphorus or to sodium, an experiment was carried out in which the sodium



Photograph by J. A. Carlile

FIG. 2. Typical corn seedlings that had received 0, 100, and 200 mg. of phosphorus, respectively, 3 times each week, photographed after 3 weeks of nutrient treatment. A. Healthy seedlings. B. Infected seedlings 16 days after inoculation.

concentration was held constant at all phosphorus levels by adding the required amounts of sodium as Na_2SO_4. The results from this experiment were comparable with those recorded in table 3, in that infection was more severe at the higher levels of phosphorus. However, seedlings that received

sodium but no phosphorus were somewhat more severely infected than those that received a deficiency of both sodium and phosphorus. These findings indicate that the differences in the host-parasite association brought about by the addition of increasing amounts of NaH_2PO_4 were due primarily to the variations in the phosphorus levels and not to the variations in sodium levels.

Influence of Potassium Supply on Host Reaction

In the third experiment, the influence of potassium supply was studied. The composition of the basic solution is given in table 1. Potassium was added as K_2SO_4 in amounts ranging from 0 to 300 mg. per 100 cc. of solution. In all solutions the nitrogen concentration was held constant at 8 mg. and the phosphorus at 10 mg. per 100 cc. of solution.

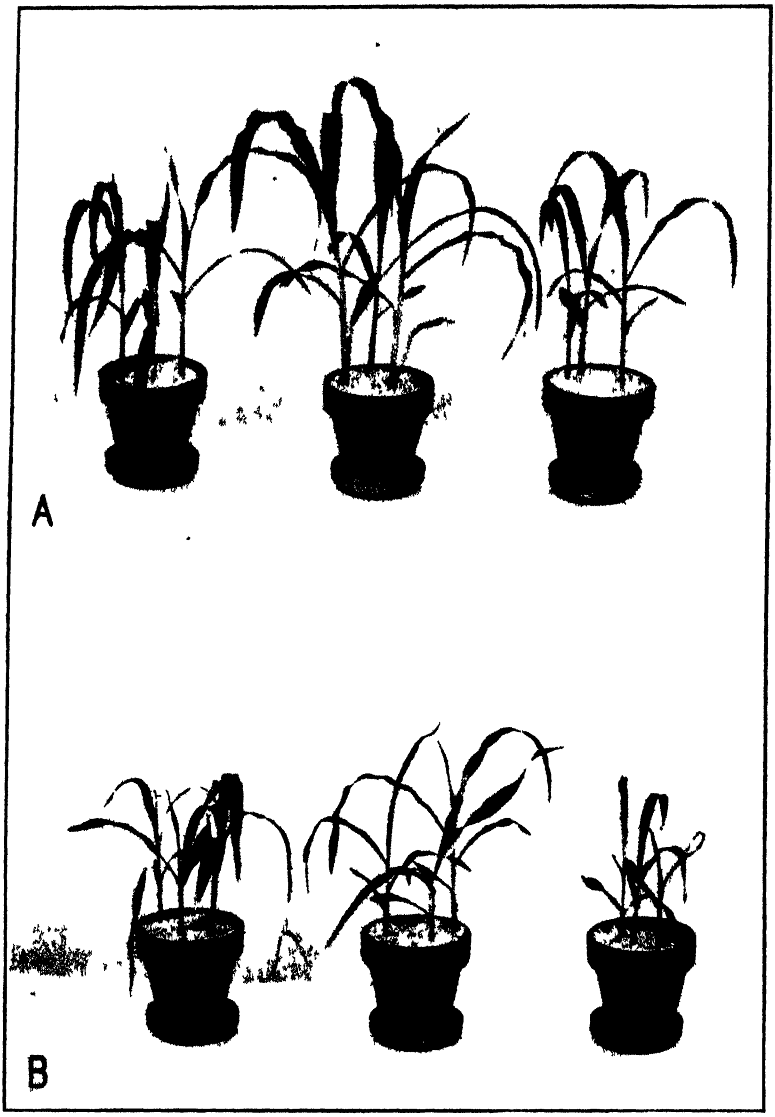
The experimental data given in table 4 show that growth of the healthy

TABLE 4.—*Influence on potassium supply on growth and reaction of corn seedlings to infection with Phytomonas stewarti*

Potassium added	Average dry weight per plant		Reaction of diseased seedlings				
			Number of leaves			Number of lesions	Index of infection
				Invaded			
	Healthy	Infected	Total	Living	Dead		
mg./100 cc.	*gm.*	*gm.*					
0	.44	.36	172	98	13	194	1.35
20	.56	.45	172	82	10	134	.95
40	.61	.39	170	66	14	105	.86
60	.67	.37	165	70	16	122	1.03
80	.59	.43	174	80	12	143	1.03
100	.61	.39	162	69	18	119	1.07
150	.58	.43	167	74	16	135	1.10
200	.49	.30	143	67	27	121	1.41
250	.47	.34	144	63	27	108	1.31
300	.41	.26	131	56	17	94	1.11

seedlings increased as the concentration of potassium increased to a level of 60 mg. per 100 cc. of solution. Growth was retarded as the concentration was increased beyond this point. Infected seedlings that had received between 20 and 150 mg. of potassium per 100 cc. of solution showed little variation in growth. Figure 3 shows representative healthy and infected seedlings that had received 0, 80, and 200 mg. of potassium 3 times each week for 3 weeks.

Plants that received no potassium showed distinct symptoms of potassium deficiency. The older leaves of healthy plants showed a necrosis, which spread from the tip along the margins to the leaf sheath. The young leaves of healthy seedlings were apparently normal, whereas the young leaves of infected seedlings showed a very marked chlorosis. Definite symptoms of toxicity appeared on all seedlings that had received high levels of potassium. The young whorls failed to develop normally, and they gradually became necrotic. The older leaves became light brown and necrotic. In all treat-



Photograph by J A Carlile

FIG. 3. Typical corn seedlings that had received 0, 80, and 200 mg. of potassium, respectively, 3 times each week, photographed after 3 weeks of treatment. A. Healthy seedlings. B. Infected seedlings 16 days after inoculation.

ments where toxicity occurred, the toxicity symptoms were more severe on the infected seedlings than on the healthy seedlings. When 200 mg. of potassium were added 3 times each week, irregular blotches developed on leaves of the infected seedlings but not on those of the healthy seedlings. The same condition with regard to necrosis of the leaf whorls was noticeable at the next higher potassium level (250 mg.). At the highest potassium level (300 mg.), more leaf whorls were injured in infected seedlings than in healthy seedlings.

The data on disease response show that potassium-deficient seedlings were very severely infected, whereas seedlings that had received 20 or 40 mg. of potassium per 100 cc. of solution were only slightly infected. However, the infection became more severe as the potassium level was raised from 40 mg. to 200 mg. These findings with regard to potassium deficiency are very striking and are apparently significant, since 2 subsequent repetitions of this experiment revealed the same relationship. The type of necrotic lesions formed as the result of inoculation appears to be similar in all treatments. Wilting of invaded leaves was more prevalent at the higher potassium levels, but none of the seedlings died as a result of infection in the 14-day period following inoculation.

DISCUSSION

The average number of necrotic lesions per leaf should serve as a satisfactory measure of the host reaction of the pathogen, since corresponding host tissues in all treatments were exposed to the bacterial culture. The infection index, as used in the experiments reported above, is an approximate measure of the number of leaf veins that necrose because of invasion by *Phytomonas stewarti*. Since the severity of infection usually was noted 10 days after inoculation, the infection index, as used here, is essentially a measure of primary infection. Seedlings with low infection indexes suffered very little secondary invasion and generally recovered.

It has been reported frequently that an actively growing host is more susceptible to disease than a slow-growing host. Trelease and Trelease (9) have shown, however, that some condition, or conditions, apparently independent of host vigor appears to determine the susceptibility of wheat to mildew (*Erysiphe graminis* DC.). A similar situation seems to exist in the host-parasite complex of bacterial wilt of maize, as shown by the experimental evidence here reported. For example, seedlings that received nitrogen or phosphorus at concentrations optimum for plant growth were more severely infected than those deprived of the element in question. On the other hand, seedlings that received small amounts of potassium grew better but were less severely infected than those deficient in potassium.

An increase in the concentration of nitrogen, phosphorus, or potassium beyond that optimum for plant growth, as judged by dry weight, increased the severity of the disease. It is probable that the excessive wilting produced by these treatments is due partly to high osmotic pressure of the solution and partly to invasion by the bacteria. The quantity of water available for the leaves apparently is reduced not only by a plugging of the tracheal tubes by the organism, but also by the increased osmotic concentration of the solution.

The effect produced by different amounts of nitrogen, phosphorus, or potassium on the reaction of corn seedlings to *Phytomonas stewarti* may be due to a change in (1) the composition of the host cell so as to alter its susceptibility to parasitic attack, (2) the rate of multiplication of the pathogen, or (3) the virulence of the pathogen within the host. From the experimental

evidence presented in this paper, it is not known which of these changes has been instrumental in producing the observed reaction.

If the nutrient solution affected the pathogen directly, the action would undoubtedly be brought about through a change in the composition of the transpiration stream. Lowry, Huggins, and Forrest (4) have shown that the mineral composition of exuded maize sap depended to some extent on the fertilizer treatment. If the nutrients added in the experiments reported here were taken up by the roots and transported through the tracheal tubes, it is evident that the pathogen was growing in a medium the composition of which varied with that of the nutrient solution. If there is a difference in the composition of tracheal sap in seedlings grown under different nutrient treatments, it might change the growth rate of the bacteria or encourage the differential development of strains that vary in pathogenicity.

It is possible that competition between host and pathogen for the available nutrients may be a factor governing the severity of the disease. When the element in question is present in low concentration, the normal processes of the host cell may utilize it to such an extent as to leave an actual deficiency of it as far as the pathogen is concerned. As the supply of the element increases, the competition between host and pathogen may develop sufficiently to overcome any resistance the host cell might normally have possessed. With high-phosphorus and high-potassium nutrition, a dynamic equilibrium apparently exists between host and pathogen. With high-nitrogen nutrition, however, the pathogen was able to kill the host.

It is of interest to note the response shown by potassium-deficient seedlings to the disease. The infected seedlings developed a pronounced chlorosis, a condition not shown by the healthy seedlings. Moreover, the disease was more severe in seedlings deficient in potassium than in those supplied with low concentrations of potassium. This difference in severity may possibly be explained as follows: The pathogen may have a low potassium requirement and may cause considerable injury when inoculated into seedlings weakened by a deficiency of potassium. However, with the addition of small amounts of potassium, the seedlings start normal growth, and in so doing may become more resistant.

SUMMARY

The influence of nitrogen, phosphorus, and potassium nutrition on the reaction of sweet-corn seedlings to *Phytomonas stewarti* was studied. Severity of infection was noted 10 days after inoculation.

Seedlings dwarfed by high concentrations of nitrogen, phosphorus, or potassium were more severely infected than those grown at concentrations more conducive to rapid growth. Seedlings deficient in either nitrogen or phosphorus were only slightly infected, whereas potassium-deficient seedlings were severely infected.

Seedlings supplied with a nitrogen-deficient solution developed small necrotic lesions but little or no wilting of invaded leaves. With high nitro-

gen, wilting was so intense that about one-half of the seedlings died within 2 weeks after inoculation.

At low phosphorus levels, infection was characterized by the formation of definite necrotic lesions, whereas at high levels infection was manifested not only by necrotic lesions but also by a dwarfing of the seedlings and a general wilting of the invaded leaves. None of the seedlings died as a result of infection.

In the potassium-deficient seedlings, infection was more severe than in seedlings receiving small amounts of potassium. The potassium level had no effect on the type of necrotic lesions formed, but high levels favored the wilting condition of invaded leaves.

It is evident, therefore, that mineral nutrition exerts some influence on the host-pathogen complex of bacterial wilt of maize. The exact changes brought about in this complex by nutrition have yet to be ascertained.

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PHYTOPATHOLOGICAL NOTES

Lightning Injury in Banana Plantations.—Spots of killed bananas, that may be from 40 to 60 feet in diameter, have been noted in large banana plantations. These areas appear after severe electric storms, and it is believed that they could be caused only by lightning. Detailed examination and study were made of 3 spots in banana plantations in the vicinity of Tela, Honduras. Two of these were observed, respectively, on September 24, 1923, and October 1, 1923, and the last one was examined on July 28, 1930. The first two spots were undoubtedly produced during a severe electric storm of September 16, 1923. The last spot observed must have resulted from a storm that occurred some 2 weeks earlier. The symptoms as described were the same in each of these areas.

The affected spots are characterized by a twisted mass of collapsed banana plants in the center of the areas and partially cooked plants that had yellowed, drooping leaves on the outer edges of the areas (Fig. 1). The outer row of plants facing the center of the area was not wholly affected; only the sides of the plants toward the center of the spot appeared scorched or had yellowed leaves. Some low-growing weeds were scorched, but most of them apparently were still in a normal state of growth. No burning was noted in the soil refuse nor was any apparent change in the soil observed.

A detailed examination of the worst affected plants in the center of the spots showed them to be entirely wilted down, as if they had been scalded. The plants had collapsed in a twisted, cooked mass. The interior of the pseudostems had the appearance of tissues that were immersed in boiling water. The entire pseudostems of the worst affected plants had a cooked appearance that extended down to the rhizomes at the ground surface. No evidence of rot was present at this early stage. The smaller suckers arising from these rhizomes were for the most part not severely affected and the rhizomes appeared to be normal. A later examination of the lightning-struck areas showed this to be true, as the suckers grew and produced normal plants, thereby filling the affected area again with healthy plants.

Plants at the outer edges of the spots were less affected and were characterized by a scorching and falling over of the upper leaves of the worst affected plants, or merely a yellowing and drooping of the leaves of plants on the extreme outer edges of the spot. The outer leaf sheaths of the most severely affected plants were slightly browned and appeared as if scalded. The young central group of developing leaves within the pseudostem of these were dried, pithy and white. This interior symptom ran through the pseudostem to the rhizome.—OTTO A. REINKING, New York State Agricultural Experiment Station, Geneva, New York.

Control of Sclerotinia and Botrytis Stem Rots of Greenhouse Tomatoes and Cucumbers.—Sclerotinia and Botrytis stem rots, Botrytis fruit rots, and

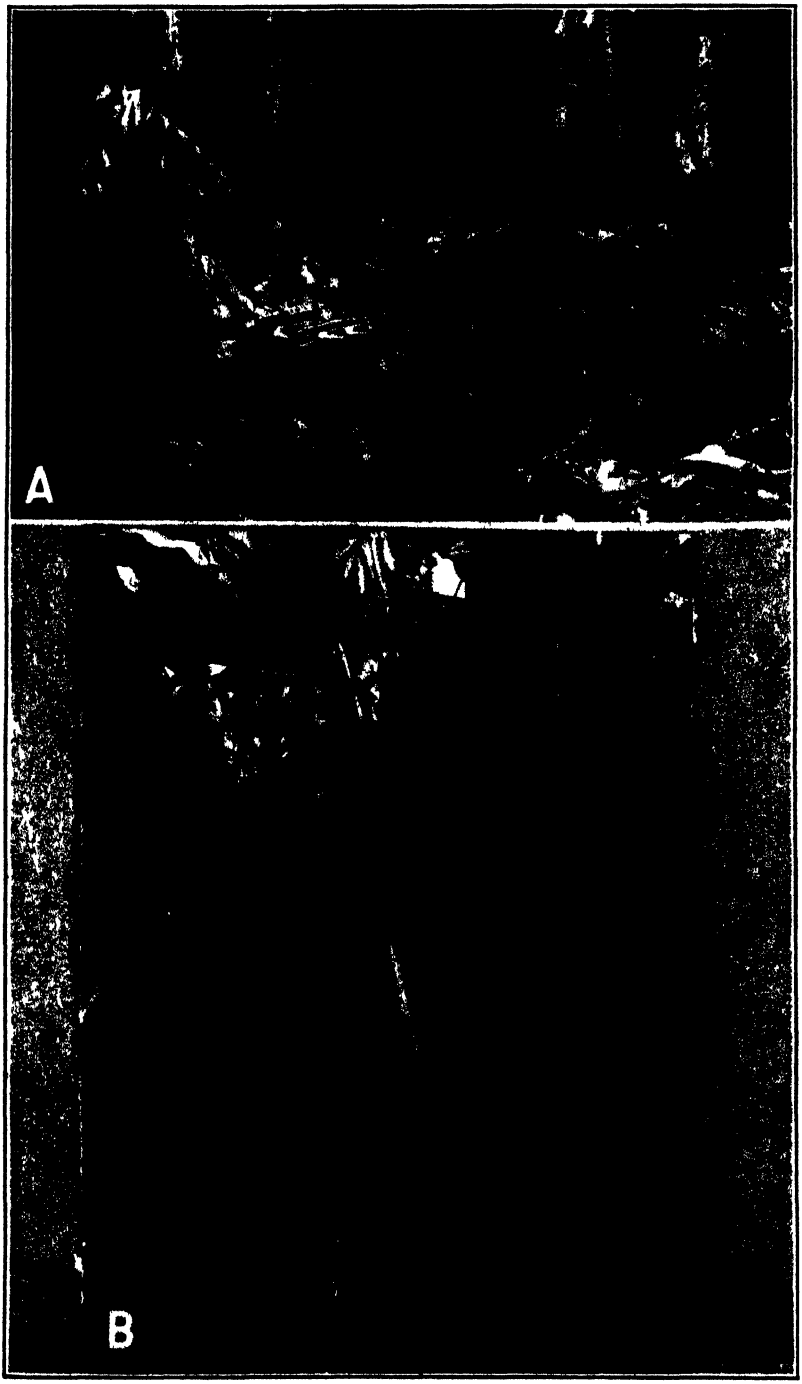

FIG. 1. Lightning injury. A. Collapsed banana plants in center of affected area. The mass of softened, twisted pseudostems is characteristic. Photographed on Sept. 24, 1923. B. Partially cooked banana plant at edge of affected area. Photographed on Oct. 1, 1923.

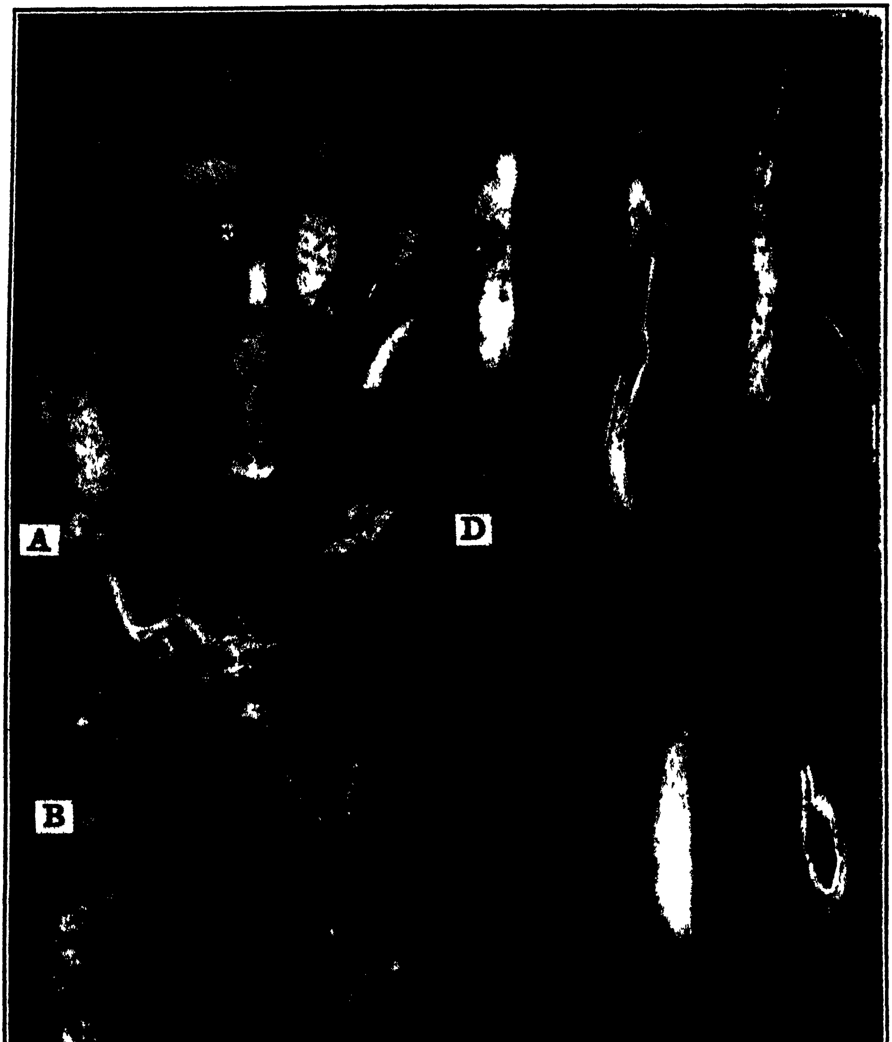

FIG. 1. A. Botrytis rot of cucumber fruit. In most instances this phase of the disease appears to have its origin from blossoms which were fertilized before coming infected. B. Blossom blight of cucumber caused by Botrytis Sp. This is a rather common stage of the disease, especially on male flowers. C. Botrytis stem end rot of tomato. Under favorable conditions losses from fruit infections may be large. D. Tomato stems infected with *Sclerotinia libertiana*. Note leaf petiole on right of picture through which the fungus entered the stem from the ground. The figure on the left became infected at point where leaf had been removed. The sclerotia in the center figure are typical. A similar disease usually without sclerotia, although small ones are sometimes found, may be caused by a *Botrytis Sp.* As a rule, an abundance of spores are visible when the disease is caused by Botrytis. Quite often the two fungi are found in the same lesion. E. Tomato stem prepared to receive the Bordeaux paste treatment. This stem was infected by Botrytis. F. The same stem after Bordeaux paste had been applied. G. Another stem, similarly diseased and treated, with Bordeaux removed one month after treat ment was applied. Stems nearly rotted through have been saved by the Bordeaux paste treatment.

blossom blight of greenhouse tomatoes and cucumbers are diseases usually considered to be of minor importance. (Fig. 1, A–D.) Occasionally, however, these troubles are of genuine commercial concern. For 2 months of the spring of 1935 damp, cloudy weather prevailed in the Chicago area and, as a result, the above diseases became so well established in a few greenhouses of tomatoes and cucumbers as to cause losses amounting to 60–75 per cent. It was estimated that total losses in Cook County averaged between 15 and 20 per cent. Although these diseases are seldom of any such importance, if they do occur, the control measures now recommended (soil sterilization and spraying plants with 4–4–50 Bordeaux mixture) are of doubtful value in saving an infected planting. We have observed that soil sterilization, although effective for the control of stem rot (timber rot), caused by *Sclerotinia libertiana* Fckl., will not prevent Botrytis fruit and stem rot from becoming quickly established in new plantings. The latter disease is usually in evidence whenever the environment and crops are suitable for its development. As noted below, spraying has been effective in a few instances.

With the above facts in mind, several methods and materials were tested with the hope of finding a suitable control for both troubles—one that could be applied effectively after the diseases had made their appearance.

Experiments were conducted in cooperation with several commercial growers and also at the University under controlled conditions. To date nothing outstandingly effective has been found for the control of Botrytis blossom blight and fruit rot on either tomatoes or cucumbers. In a few tests sulphur sprays and dusts appeared effective on both crops, as did also basic copper sulphate and Bordeaux mixture sprays. Subsequent tests, however, indicate that these treatments are not to be relied upon; in fact, the Bordeaux spray is almost sure to injure cucumbers. Fortunately, the fruit rot and blossom blight phases of the Botrytis disease are not the most important. The greatest losses are sustained from stem rots caused by the two fungi.

Of the several different methods tried as possible control measures, a Bordeaux mixture made of 1 part copper sulphate, 2 parts lime, and enough water to make a thick paste, has given nearly perfect control of stem rots after they had become well established. The most effective method of application is to gouge out the infected tissue with a knife or stick and fill the cavity thus formed with the paste. (Fig. 1, E–G.) Plants nearly rotted through, when thus treated, have been saved and continued to develop and produce apparently normal crops. The treatment has been equally effective on both tomatoes and cucumbers, but, in a few instances, it has caused injury to cucumbers, resulting in the yellowing of some of the leaves.—K. J. KADOW, H. W. ANDERSON, and S. L. HOPPERSTEAD, Department of Horticulture, University of Illinois, Urbana, Illinois.

A Root and Collar Disease of Pine Seedlings Caused by Sphaeropsis ellisii.—During the investigation of *Phytophthora cinnamomi* root disease of *Pinus resinosa* seedlings, many of the 3- to 5-year-old trees in two Maryland nurser-

ies were found to be dying from a root rot characterized by symptoms differing from those typical of Phytophthora rot. Resembling Phytophthora rot to the extent that the area attacked often extended from the collar to a point below the soil line, this rot was, however, not characterized by exudation of resin nor by the infiltration of resin in the wood, which commonly follows *Phytophthora* attack on this host. The invaded bark tissue was deep red, with black streaks continuing into the xylem and often throughout the entire stele. Five- or 6-year-old *Pinus strobus* trees, showing similar symptoms, were received from a plantation in Wisconsin.

A fungus, black in culture, was isolated from the discolored tissue of both the *Pinus resinosa* and the *P. strobus* trees showing the symptoms mentioned. Sections of infected root and collar tissue were placed in a damp chamber, where typical *Sphaeropsis* pycnidia and spores (averaging 36 x 12 μ) developed within a few days. Neil E. Stevens examined the fungus, both in culture and on the host, and identified it as *Sphaeropsis ellisii* (Sacc.). *S. ellisii* has been previously reported as a common parasite on a large number of coniferous hosts, but always as the cause of various twig die-back diseases.

Single-spore isolations of the fungus from *Pinus resinosa* were used in inoculations in comparison with isolates of *P. cinnamomi* obtained from diseased roots of seedlings of the same species and considered truly pathogenic on them.[1] Inoculations were made by inserting rice grains on which the fungus was growing in cuts extending through the bark to the cambium of 3-year-old *P. resinosa* seedlings. Check inoculations were made by inoculating with sterile rice and with cultures of *Sclerotium bataticola* and *Pestalozzia funerea,* also isolated from diseased *P. resinosa* roots. Immediately after it was inoculated, the wound was covered with dry cotton and linen tape.

In the first test half of the seedlings were inoculated at the soil line and the remainder an inch below it. Within 20 days the 12 seedlings inoculated with *Sphaeropsis*, the 40 inoculated with 2 lines of *Phytophthora,* and 5 of the 20 inoculated with *Pestalozzia* had died, whereas the 8 inoculated with *Sclerotium* and 20 with sterile rice remained healthy. The place of inoculation apparently did not influence the result, since rot of both root and collar resulted from each.

In a second test 6 trees each were inoculated at the collar with cultures of *Phytophthora, Sphaeropsis,* and *Pestalozzia.* Bits of rice on which *Phytophthora* and *Pestalozzia* were growing were inoculated into small cuts, while the rice on which *Sphaeropsis* was growing was merely placed in contact with the nonwounded bark. Inoculations with sterile rice were used as a check. Over a period of 18 days, during which *P. cinnamomi* invaded tissue an average of 7.5 cm. in each direction from the collar, the isolate of *S. ellisii* progressed an average of 5,5 cm. down into the roots and 12 cm. up the stem. In both cases all inoculated seedlings were killed. Three of the 6 seedlings inoculated with *Pestalozzia* were killed, with the fungus apparently present up to 8 cm. in the stem but not in the roots at all. All checks remained

[1] Jackson, L. W. R., and B. S. Crandall. A Phytophthora root and collar rot of *Pinus resinosa* seedlings. (Abstract) Phytopath. 25: 22. 1935.

healthy. In all the *Sphaeropsis* and *Phytophthora* inoculations, symptoms were produced, typical of those found associated with the respective fungi in the field and the fungus reisolated.—BOWEN S. CRANDALL, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

The Propagation of Tobacco Plants from Root Cuttings.—During the summer of 1936 a crop of tobacco (*Nicotiana tabacum*) of the variety "Virginian" was grown in the experimental garden of the Virus Research Station here. The plants were infected with a strain of Tobacco Virus No. 1 kindly supplied by the Department of Animal and Plant Pathology of the Rockefeller Institute, Princeton, N. J. They were harvested in October, 1936, the ground being dug over and narcissus bulbs planted in connection with an-

FIG. 1. Fragment of the root of an old tobacco plant from which has grown a new plant.

other investigation. At the end of July, this year, it was noticed that here and there on the plot plants of tobacco had made their appearance, the foliage of the bulbs having died down. The tobacco leaves all showed symptoms of ordinary tobacco mosaic (Tobacco Virus No. 1). Since there is little sound evidence that this virus is transmitted in the seed, a careful examination of

the diseased plants was made to discover if they were in fact seedlings infected through the seed. When the plants were dug up it was found that they had developed from portions of roots inadvertently left in the ground when it had been cleared in the previous autumn. It would appear that the tobacco plant can readily propagate itself by root cuttings and that under mild climatic conditions, at least, this may constitute a possible source of infection for the next year's crop. The question of the persistence or not of the virus in leaves and plant débris may be of less importance under these conditions than the possibility of the appearance of actual plants from portions of diseased roots of the previous crop, analogous to the "Groundkeepers" found in potato fields.

Figure 1 shows the appearance of one of the roots with its attached plant. —JOHN CALDWELL and A. L. JAMES, Department of Botany, University College, Exeter.

NOTICE TO MEMBERS OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY CONCERNING DESCRIPTION AND NOMENCLATURE OF PLANT VIRUSES

It was voted at Indianapolis to have as a special item on the next annual program of the Society a discussion of the description and nomenclature of plant viruses. The Council has taken the following action looking toward the development of this important feature of the program.

The following committee has been appointed to arrange for the virus program at Richmond, Virginia: James Johnson, Chairman; L. O. Kunkel, C. W. Bennett, Eubanks Carsner, H. H. McKinney, E. S. Shultz, W. D. Valleau.

The following resolution, passed by the Council, will be presented to the Society for its consideration at Richmond:

The Council recommends to the Society for its favorable consideration at the Richmond meeting the following: Resolved that the American Phytopathological Society express to the International Committee on Description and Nomenclature of Plant Viruses appreciation of the work they have done and confidence in the plan outlined in their "Illustration of Proposed System of Nomenclature for Plant Viruses," which was presented in mimeographed form at the Sixth International Botanical Congress (Amsterdam, 1935) and adopted in principle, the committee being empowered to continue its considerations and establish an acceptable system of virus nomenclature. It is further the sense of this Society that it is desirable for individual workers to cooperate as much as possible with the International Committee on Descriptions and Nomenclature of Plant Viruses and in so far as feasible to refrain from adopting any new system of nomenclature until this committee has had the opportunity to make its next report.

Drs. Johnson and Kunkel extend to all who are interested in the description and nomenclature of plant viruses a cordial invitation to make criticisms

or suggestions for the betterment of the plan[1] now under consideration by the International Committee, which will be the basis for the discussion at Richmond.

R. S. KIRBY, Secretary.

BOOK REVIEW

KENNETH M. SMITH, D.SC., PH.D. *A Textbook of Plant Virus Diseases.* Pp. 615, 101 figs. London. J. and A. Churchill, Ltd. 1937. 21 s.

This book is the outcome of a serious effort to bring together in a handy volume the scattered world literature on virus diseases of plants. The author himself has been occupied for about 18 years with all the phases of plant virus work. In his earlier book: "*Recent Advances in the Study of Plant Viruses*" he has given an excellent review of problems and methods, together with a short survey of special viroses. The new book is entirely devoted to the special treatment of plant viroses. It is an extensive compilation and must have entailed a vast amount of work. There is no other book of its kind in existence. The reviewer does not like the title. It is not a text-book but a reference work. It is really a kind of dictionary of plant viruses with descriptions of the various diseases they cause. Nor does the reviewer agree with the author's attitude in wanting to make a special science of "Virology." We have already enough water-tight compartments; the study of viroses of plants is the last that can be circumscribed. Already, it needs botanists, entomologists, biochemists, etc! Surely, it is sufficient to include it in Plant Pathology.

The scheme of classification followed is a modification of and an improvement on that suggested by James Johnson, of Wisconsin. Each virus is grouped according to its first-described or most important host plant, and the hosts are arranged according to the families to which they belong. Under each particular host plant, for which the Latin generic name is used, the viruses are numbered consecutively 1, 2, 3, etc., and the strains of certain viruses are denoted 1a, 1b, 1c, etc. The properties of each virus *in vitro* (so far as they are known at present), its mode of transmission, and the diseases it causes are described. The insect vectors are treated in some detail in a separate section of the book.

Special mention should be made of the appendix, giving the most characteristic symptoms of the various virus diseases on the more important host plants. It helps the student to arrive at a preliminary diagnosis of a suspected virus disease and also enables him, by means of the page references, to find in the book the information on all the viruses that attack any one particular plant. By this means, also, the disadvantage of the classification of the viruses, rather than the diseases they produce, will be partly overcome.

In many respects this book is excellent. Its style is clear, and it will be of value for many research workers and teachers of plant pathology. It is well illustrated, and printed on good paper. Some critical remarks must, however, be made and they will be restricted to Chapter VI, in which the viroses of the potato are treated in detail. Most of the descriptions are good, but the historical sequence of the discoveries shows some gaps. This may be due to the fact that the author, in spite of the existence of excellent abstracting journals, such as the Review of Applied Mycology and Biological Abstracts, has an incomplete knowledge of such literature as is not English or American.

In the introduction to Chapter VI, W. A. Orton, together with Quanjer and Appel, is considered the father of modern plant-virus work. In the same metaphorical language, however, Appel and Orton should really be regarded as godfathers. Appel stood godfather to the name "Blattrollkrankheit," Orton to the name "Potato Mosaic." But what is a name when it is not known whether the godchild is a fungal, bacterial, or nonparasitic disease?

What the author says of Solanum virus 1 Orton, sufficiently proves that it is not possible at this date to determine which virosis of the potato Orton had before him. Solanum virus 1 Orton is wrongly considered as being identical with Quanjer's acronecrosis virus and his interveinal mosaic virus. Köhler's ring mosaic virus is not considered at all. That the difference in severity of the symptoms produced by the strains

[1] Johnson, James. The nomenclature of plant viruses. Proc. Sixth International Bot. Cong. (Amsterdam) 2: 193–195. 1936.

of Solanum virus 1 would be less distinct on potatoes than on tobacco is not the view of the present reviewer. The preponderating English-American orientation of the author appears also from the fact that he appears to be unaware that only a restricted number of commercial potato stocks contain viruses of the "healthy potato virus" type on the continent of Europe.

Solanum virus 2 Orton is mistakenly considered to be synonymous with Atanasoff's stipple-streak virus and Quanjer's Acropetal Necrosis Virus. In contrast to Orton's Solanum virus 2 the virus described by Atanasoff and Quanjer produces internal necrosis in the tubers. Köhler's Strichelvirus is not mentioned in this connection. Solanum virus 2 is not a simple one but is rather an association of different viruses that must be distinguished.

Solanum virus 4 Murphy has a pathological effect that does not appear only as primary symptoms, for secondary symptoms also are known, to which Oortwijn Botjes first called attention.

Photomicrographs of the histopathological effect of viruses 1 and 2 are given; but an illustration of the classical phloem-necrosis, described in 1913 by Quanjer, is lacking. Although, in 1920, Oortwijn Botjes was the first to describe the transmission of Solanum virus 14 by the peach aphis, the name of K. M. Smith and the year 1929 alone are mentioned in this connection. Elze, in 1927 (*i.e.*, 4 years earlier than K. M. Smith), was the first to prove and describe the obligatory connection between this virus and its vector and to determinate the "waiting" time. Elze was also the first to give exact data on the transmission of this virus with true potato seed. Thung's fundamental work of 1928 on the physiological aspects of the leaf-roll disease is not mentioned.

In a book of this kind it is perhaps inevitable that omissions and errors will be detected, such as those just mentioned; but, though unfortunate, they do not detract unduly from the general usefulness of the text and they can be rectified in a future edition.—H. M. QUANJER, Instituut voor Phytopathologie, Wageningen, The Netherlands.

RHIZOCTONIA SHEATH SPOT OF RICE

T. C. RYKER AND F. S. GOOCH

(Accepted for publication Dec. 8, 1937)

A disease of rice characterized by the presence of rather large, light-color spots on the leaf sheaths has been known in certain Oriental countries for a number of years. In Louisiana, a disease either identical or at least very similar to the Oriental sheath spot was apparently first observed in 1933. In the Orient, this disease is said to be caused by Rhizoctonia-like fungi, either *Rhizoctonia solani* Kühn or *Hypochnus sasakii* Shirai.

In Louisiana, probably the first isolations from sheath spot lesions were made by D. E. Ellis[1] at the Louisiana Agricultural Experiment Station in 1933. He isolated a reddish *Rhizoctonia* with which he obtained typical sheath spots by inoculation. Later, however, in 1934, Tullis (17) described the sheath spot, as it occurred in Louisiana, Arkansas, and Texas, and claimed that it was caused by a species of *Trichoderma*.

Since 1933 a study of the disease and the organisms associated with it has been made in Louisiana.

SYMPTOMS, OCCURRENCE, AND IMPORTANCE OF THE DISEASE

The sheath spot of rice is characterized by the presence of rather large, necrotic areas or spots confined almost entirely to the leaf sheaths. Spots do occur occasionally on leaves, but none has been observed on stems. A spot first appears as a reddish-brown discoloration that quickly enlarges and assumes an elliptical shape. In older stages it has a bleached, straw-color center and a somewhat broad, reddish-brown margin. The deepest color is next to the center. The lesions may further enlarge and coalesce to form irregular discolored areas. The spots are usually from 1 to 3 cm. long, though they may reach a length of 10 cm. or more. The width is usually about one-half the length. The spots usually occur above the water line and quite frequently just below the ligule. They may occur on any of the leaf sheaths, though they usually are confined to the lower ones. Sclerotia have never been observed either on or between diseased sheaths. Typical leaf-spot lesions are shown in figure 1.

The disease first appears during the latter part of July and becomes increasingly prevalent thereafter until September, with little development thereafter. The rice plants are well developed on first appearance of the disease and are usually well headed by the time of maximum development. The prevalence of the disease varies with the field and with the location in the field. It is usually more severe along the levees and in other places where the plant growth is more dense, although it may occur on isolated plants. All the commercial varieties appear equally susceptible. In 1936 and 1937 the disease was more prevalent in the prairie region of south-

[1] From notes on file in Department of Botany, Louisiana State University.

western Louisiana than in the Bayou Tèche and Mississippi River sections. In an extensive survey made in 1936, the percentage of infection in different fields varied from a trace to as high as 50 per cent. Sheath spotting also has been found on barnyard grass, *Echinochloa crusgalli,* present in rice fields.

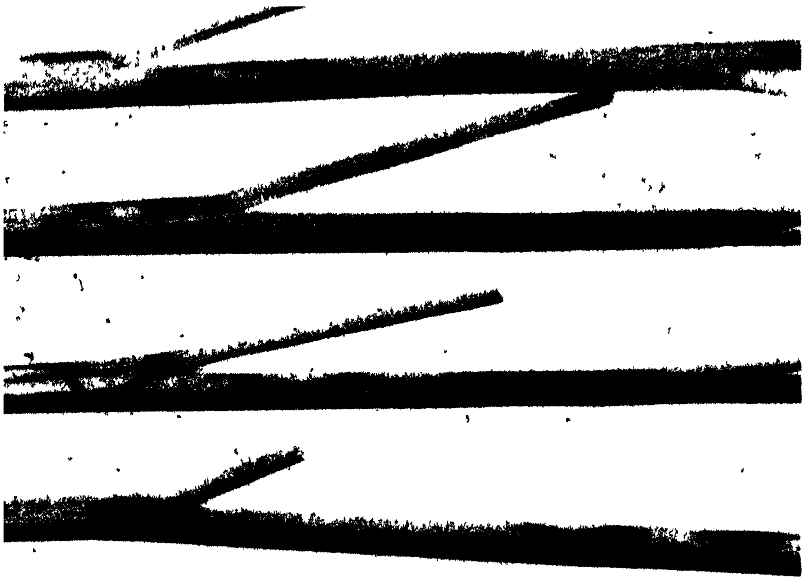

FIG. 1. Sheath spot lesions on Rexora rice.

The disease appears to be of no serious economic importance in Louisiana. Its importance lies in the fact that the functioning of certain of the leaves is impaired at heading time, a crucial period in development of the rice plant. Moreover, the infected plants may lose some of the support that the sheaths give to the stem. On the other hand, in Japan (6, 9, 15) and some of the other countries of the Eastern Hemisphere, the sheath spot of rice has been considered to be one of the more serious rice diseases, for, in addition to sheath spotting, the seedlings are affected.

THE CAUSAL ORGANISM

Platings made of diseased sheath tissue in Louisiana have invariably shown the presence of a strain of *Rhizoctonia* characterized by the production of salmon-color sclerotial masses in culture. The organism, as grown on artificial media, is shown in figure 2. It has been cultured successfully by plating small pieces of diseased tissue on plain agar, or bean-pod agar, after thoroughly washing with tap water and rinsing in sterile water. The use of surface disinfectants was not so successful, as the tissue is readily penetrated and the fungus is apparently quite sensitive to disinfectants.

In order to check closely on the causal organism, extensive collections of diseased plants were made in 1936 and 1937, and more than 1,600 platings

were made of material from 30 different collections from Louisiana and Texas. Pure cultures of *Rhizoctonia* were obtained from more than 1,000 of these. In all but 3 collections the strain bearing salmon-color sclerotia was the only one isolated. In 2 of the 3 exceptions a strain of *R. solani* with larger sclerotia and a strain of *R. zeae* (18) were isolated, along with the usual salmon-color one. In the third instance, all isolates were typical of *R. solani* and quite similar to sheath-spot isolates obtained from China and the Philippine Islands. This last collection was made in a small rice plot

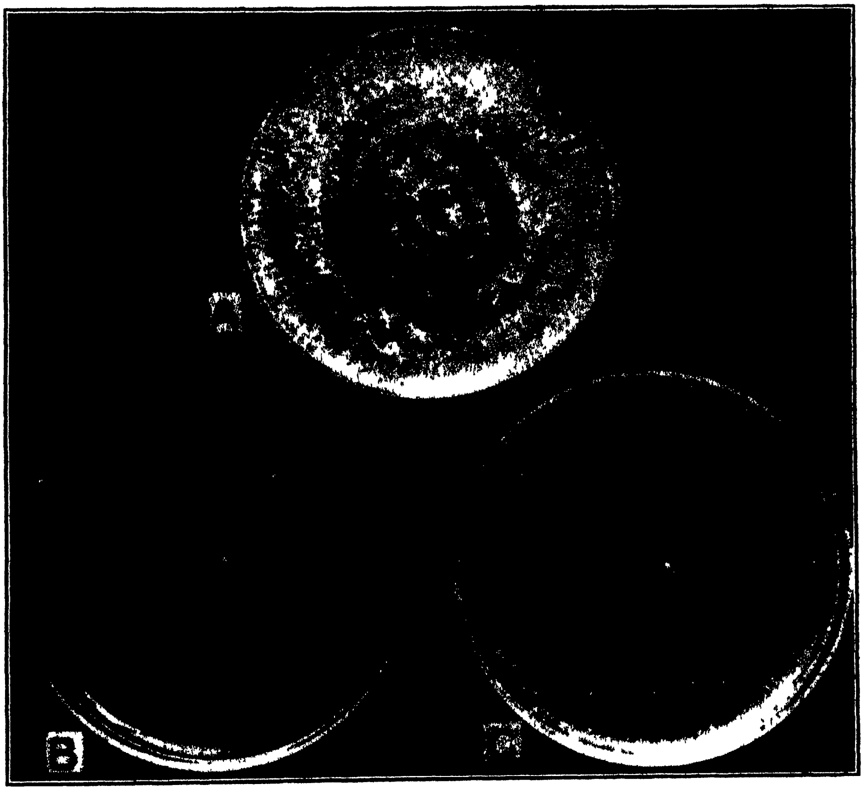

FIG. 2. Cultures of *Rhizoctonia oryzae* on different media incubated for fifteen days at 28° C. A. Bean-pod agar. B. Potato-dextrose agar. C. Cornmeal agar.

near the greenhouse in Baton Rouge, far removed from any rice field. It was further observed in this plot that sclerotia were produced between the diseased sheaths, a characteristic said to be true of the disease in the Orient. A comparison of the local isolates (1, 26, and 55) of *R. solani* with one isolate from China (104) are shown in figure 3.

TAXONOMY

A study of the literature indicates that the *Rhizoctonia* ordinarily causing the leaf-sheath disease in Louisiana has not been described, and that it is different from the organisms said to cause the same or a similar disease in the Orient.

The Oriental sheath spot is known in Japan as "Mongare byô" and has been intensively studied by workers in that country. The first report of its incidence was made in 1910 by Miyake (11), who described the causal fungus as *Sclerotium irregulare* sp. nov. However, prior to that, according to Matsumoto *et al.* (9), the fungus was collected by Sasaki on the leaves of camphor trees and was subsequently described by Shirai (16) in 1906 as *Hypochnus sasakii.* Sawada (14) in 1912, according to Matsu-

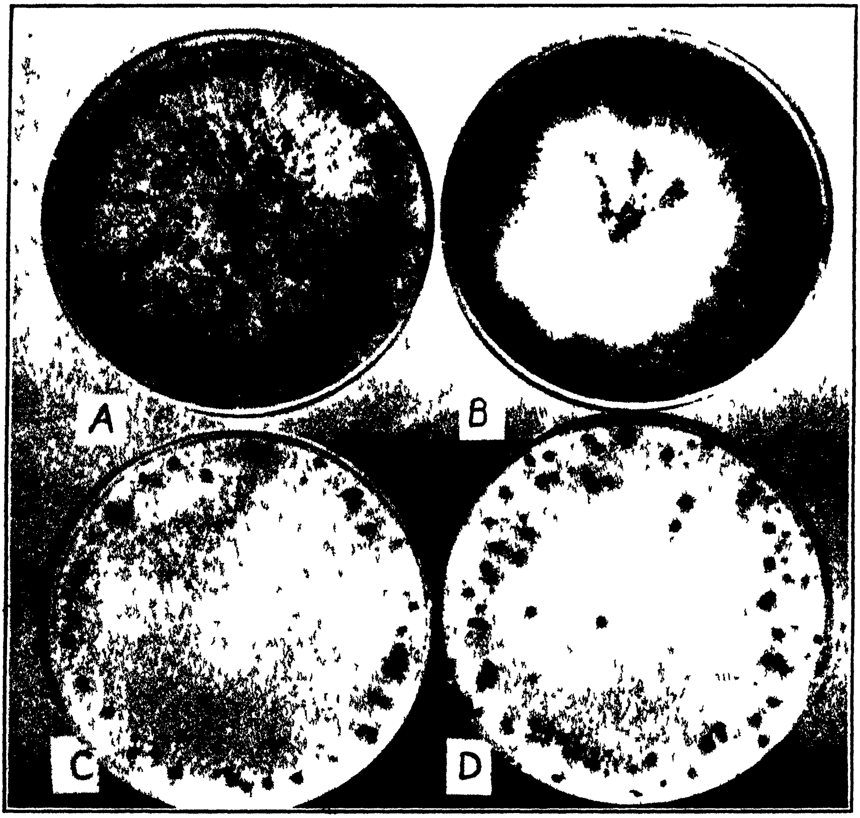

FIG. 3. Cultures of *Rhizoctonia solani* capable of producing sheath spots on rice, on potato-dextrose agar, incubated for ten days at 28° C. A. Isolate 1. B. Isolate 104. C. Isolate 26. D. Isolate 55.

moto *et al.* (9) and Wei (19) found a similar fungus on rice in Formosa and, following cross-inoculation tests, stated that *Sclerotium irregulare* Miyake on rice plants was the same as *Hypochnus sasakii* Shirai and adopted the latter name for the fungus. Sakurai (15) described 4 diseases of rice caused by sclerotia-forming fungi, one of which was that caused by *H. sasakii.* He stated that *H. sasakii* attacked the plants from June to October, producing irregular brown spots on the leaf sheaths and occasionally on the blades, and that brown sclerotia were formed on the spots or between the leaf sheaths and the stem.

In 1926, Palo (12) published his studies on a Rhizoctonia disease of rice

in the Philippines, in which sheath spotting was the typical symptom. He considered the causal organism as a strain of *Rhizoctonia solani* and similar culturally to strains of *R. solani* found on other plants in the Philippines. He also observed that the organism affected rice seedlings. Palo's fungus was later studied by Endo (2, 3, 4) in Japan and was compared with *Hypochnus sasakii.* Endo found these sufficiently alike culturally and pathogenically to be considered identical. He (3) further compared them with a strain of *R. solani* from Europe and found that the latter differed from *H. sasakii* in various respects. Workers in Japan have shown that the rice *Rhizoctonia* has a wide host range. In 1930, Ikata and Hitomi (6) described the perfect stage of the rice *Rhizoctonia* on infected leaves and sheaths of rice. They stated that the formation of basidiospores occurred at night during August and September.

In 1928, Gadd and Bertus (5) reported their studies on the diseases of *Vigna oligosperma* and other plants in Ceylon caused by *Corticium vagum* B. and C. They studied isolates from various sources, including one from sheath-spotted tissue of rice, and found that the various isolates were quite similar culturally and pathogenically. The Rhizoctonia disease of rice was further studied in India by Park and Bertus (13), and the fungus was considered by them to be *Rhizoctonia solani.* From their description, the disease and the causal organism appear to be the same as those occurring in Japan and the Philippines.

Wei (19) described the disease that occurs in China as a Rhizoctonia sheath blight and the causal organism as *R. solani.* His studies indicate that the organism he studied was the same as the Japanese strain described as *Hypochnus sasakii.* He compared the descriptions of the perfect stages given by Sawada (14) and Ikata and Hitomi (6) with that of *Corticium vagum* by Burt (1) and considered the differences insufficient to warrant species distinction.

Matsumoto and coworkers (7, 8, 9, 10) in Formosa have studied the physiology, parasitism, and taxonomy of the fungi generally referred to as *Hypochnus sasakii.* Matsumoto (7) states that a comparison of the perfect stages, when considered alone, does not show sufficient differences to justify specific separation, but these, combined with the vegetative differences, are sufficient to regard *Corticium* (*Hypochnus*) *sasakii* as distinct from *C. vagum.*

From data and observations cited in the literature and also from studies of isolates of the Oriental fungus obtained through the courtesy of Dr. C. T. Wei in China and Dr. G. O. Ocfemia in the Philippines, it seems evident that the causal fungus studied in the Orient is a large, sclerotial strain of *Rhizoctonia solani.* The Louisiana fungus, on the other hand, is distinctly different, and cannot be considered a strain of *R. solani*, despite the fact that the diseases produced on the rice plant are essentially indistinguishable. The fact that certain strains of *R. solani* and *R. zeae* have been isolated from sheath-spot material in Louisiana and are able to produce typical sheath

spotting, as will subsequently be shown, indicates the similarity of pathogenic behavior of various species of *Rhizoctonia.*

The Louisiana fungus is definitely a species of *Rhizoctonia.* The method of branching is characteristic, and a septum occurs in each branch a short distance from the main filament. Sclerotial formation, however, is quite different from that occurring with *Rhizoctonia solani.* The filaments branch separately and anastomose, finally forming sclerotial masses of hyphae with short, barrel-shape cells. Mycelium branching and sclerotia formation are shown in figure 4.

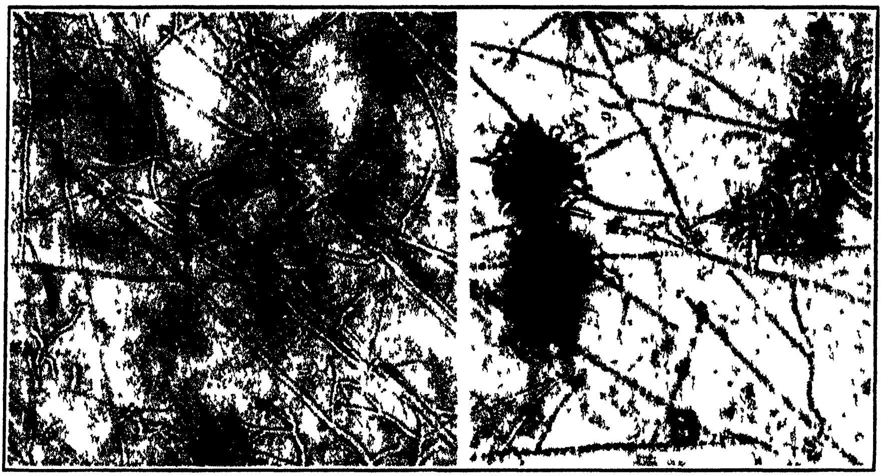

FIG. 4. *Rhizoctonia oryzae.* A. Mycelial branching in cultures three days old. B. Sclerotial formation in cultures six days old.

The sclerotial masses are of indefinite size and shape. Quite frequently they are formed along main hyphal branches, giving a crow's-foot pattern. The color of the sclerotial cell masses varies somewhat with the substrate but is a shade of salmon. According to the Maerz and Paul[2] color standard, the color is 10–B–7 on dried bean-pod-decoction agar, 10–C–6 on cornmeal-decoction agar, and 10–B–6 (sugarcane) on potato-dextrose agar. The mycelial characters also vary somewhat with the substrate. On potato-dextrose agar there is considerable aerial mycelium, there being almost none on the other 2 agars. The mycelium grows very rapidly, covering the medium in a 9 cm. Petri dish in 2 days, incubated at 32° C. Sclerotial masses usually are formed in from 5 to 7 days. No perfect stage is known for the fungus.

The fungus apparently is a new species and is described as follows:

Rhizoctonia oryzae, sp. nov. Mycelium superficial and submerged in culture, hyaline to white, granulate when young, gradually becoming clear when old. The main mycelial strands are 6 to 10 μ in width, branching at an acute angle with a slight constriction at the point of branching and with a septation a short distance from the point of constriction. The main hypha is also septate a short distance above the branch. Later,

[2] Maerz, A., and M. R. Paul. Dictionary of Color. McGraw-Hill Book Co., New York, N. Y. 1930.

short-celled, much-branched hyphae emerge at right angles from the main branches and ramify through the substrate. Certain of these after a few days develop much-thickened, short hyphae with barrel-shape cells. These hyphae intertwine and anastomose, forming masses of sclerotial cells or pseudo-sclerotia of various sizes and shape. Sclerotial masses are salmon color. Mycelium superficial and internal in leaf-sheath tissue, principally the latter. Sclerotial masses not formed on the host. Produces, in Louisiana, a sheath spot of rice, *Oryzae sativa* L. Type material deposited in Bureau of Plant Industry collections, Washington, D. C.

Isolates Studied. In making the study of sheath spot of rice, it seemed advisable to include a number of isolates of *Rhizoctonia solani* and *R. zeae.* The isolates that have been compared in the investigational work are listed in table 1.

TABLE 1.—*Isolates of Rhizoctonia made by several workers and studied by the writers in their investigation of sheath spot of rice*

Isolate	Species	Isolation: Source	Isolation: Date
1	*R. solani*	Rice seedling, D. E. Ellis	1933
2	*R. solani*	Bean plant, L. H. Person	1931
5	*R. solani*	Sheath spot, F. S. Gooch	1935
104	*R. solani*	Sheath spot, C. T. Wei[a]	
107, 108, 109	*R. solani*	Sheath spot, G. O. Ocfemia[b]	1937
25, 26, 55	*R. solani*	Sheath spot, T. C. Ryker	1936
21, 22	*R. zeae*	Sheath spot, T. C. Ryker	1936
3, 4, 9, 20, 27, 33, 34, 40, 41, 45, 46, 47, 52, 53	*R. oryzae*	Sheath spot, T. C. Ryker	1936
110	*R. oryzae*	Sheath spot, T. C. Ryker	1937

[a] Originally isolated by C. T. Wei, University of Nanking, Nanking, China, from sheath spot of rice, and obtained from E. L. LeClerg, University of Minnesota.
[b] Philippine Islands.

INOCULATION TESTS WITH TRICHODERMA

Since sheath spotting of rice had been attributed to *Trichoderma* (18), a number of attempts were made to induce infection with this fungus. In these tests isolates of *Trichoderma* obtained from rice and from other plants were used. Rice plants were inoculated by spraying with spore suspension and by placing the fungus mycelium on the host tissue. In no case was sheath spotting induced.

INOCULATION TESTS WITH RHIZOCTONIA

To obtain information on the pathogenicity of different isolates of *Rhizoctonia*, many inoculation tests have been conducted. Two methods of inoculation have been used. In one, the inoculum was placed on or in the soil in which rice plants were growing, while in the other the inoculum was placed between the leaf blade and the stem near the ligule. In the latter case the inoculum consisted of the fungus mat formed on bean-pod infusion from which most of the nutrient material had been removed by rinsing with sterile water.

Experiment I. A test was conducted to compare and test the pathogenicity of a strain of *Rhizoctonia solani* (isolate 1) originally obtained from a rice seedling, and one of *R. oryzae* (isolate 4). Four-gallon pots were filled

with soil and sterilized with steam. Pure cultures of the organisms on an oat-wheat medium were mixed with the soil and then planted with rice of the Blue Rose variety. Two pots were planted with each isolate and 2 kept for controls. The seeds were planted on May 6, 1936.

On August 11, typical sheath spots were appearing on the plants in the pots inoculated with *Rhizoctonia oryzae*. By August 11, 70 of the 100 shoots in the 2 pots had well-developed spots, all above the water line. Plants in the pots inoculated with the isolate of *R. solani* developed no spots, though mycelium was present on the lower leaf sheaths. The controls bore no spots.

This experiment was repeated; pots were planted on August 15 and plants were grown to maturity, heading about the middle of October. No sheath spots developed from inoculation with either of the 2 *Rhizoctonia* isolates. It is possible that the low temperature was responsible for the lack of infection.

Experiment II. The same cultures of *Rhizoctonia solani* (isolate 1) and *R. oryzae* (isolate 4) were used in another test. Full-grown Blue Rose plants growing in 4-gallon pots were inoculated the first week in August by placing the oat-wheat inoculum on the soil between the plants. Two pots were inoculated with each of the 2 isolates and 2 were treated with sterile oat-wheat mixture for controls. After inoculation, half of the pots were placed in a moist chamber for 33 hours, while the others were left uncovered. No sheath spots developed on plants inoculated with the *R. solani* isolate or on the controls. Sheath spots were present on the 5th day on plants inoculated with *R. oryzae* and placed in the moist chamber, and, after 6 days, on the plants not so treated. Of the 69 shoots in the 2 pots, 70 per cent showed definite spots. The sheath-spot lesions developed above the water line and quite commonly just below the ligule.

Experiment III. Another test was conducted to compare the pathogenicity of various *Rhizoctonia* cultures by the direct-inoculation method. Two cultures of *R. solani* (isolates 1 and 5) and 3 of *R. oryzae* (isolates 3, 4, and 9) were used. From 10 to 13 well-developed rice plants (Fortuna variety) growing in pots were inoculated with each isolate by placing bits of the inoculum in the leaf axis near the ligule. After inoculation the pots were placed in a moist chamber at 32° to 35° C. for 31 hours and then removed to an outdoor cage. At the time of removal from the moist chamber in many cases the sheath tissue at the point of inoculation had a water-soaked appearance, and 24 hours later these areas showed the typical symptoms of sheath spot. At the end of 5 days, 100 per cent of the plants inoculated with the *R. oryzae* isolates had sheath spots, 6 of the 10 inoculated with isolate 1 had spots, while no spots developed on the controls, or on those inoculated with isolate 5. Infection usually occurred on the sheath below the point of inoculation and usually on the inner epidermis of the sheath. Only rarely did infection take place through the outer epidermis, even though the inoculum was in direct contact with it.

The above experiment shows that certain strains of *Rhizoctonia solani*,

under favorable conditions, are able to produce sheath spot symptoms similar to those produced by *R. oryzae.*

Experiment IV. In the foregoing experiments only a few isolates of *Rhizoctonia oryzae* were used. It seemed desirable to determine the pathogenicity of a large number of sheath-spot cultures in order to find out whether isolates of *R. oryzae* varied to any extent and also to compare them with isolates of other species of *Rhizoctonia* isolated from sheath-spot tissue. Ten cultures of *R. oryzae* (isolates 20, 27, 33, 34, 40, 41, 45, 46, 47, 52), 2 cultures of *R. zeae* (isolates 21, 22), and 2 cultures of *R. solani* (isolates 25, 26) were used. This experiment was run in 2 series, being started on August 25 and August 27, 1936, respectively.

In the first series, rice plants in pots were inoculated by placing bits of mycelium back of the leaf sheaths. After inoculation the pots were placed in a moist chamber at a temperature of 32° to 35° C. for a period of 30 hours and then removed to an outdoor cage. In series 2, the oat-wheat inocu-

TABLE 2.—*Pathogenicity on rice of a number of sheath spot isolates when tissue was inoculated directly and when the inoculum was placed on the soil surface*

Isolate	Method of inoculation	Variety	Total number shoots	Percentage with sheath spots
20	Direct	Fortuna	12	41.7
	Soil	Blue Rose	29	68.9
21	Direct	Fortuna	13	84.6
	Soil	Blue Rose	33	0.0
22	Direct	Fortuna	10	100.0
	Soil	Blue Rose	22	0.0
25	Direct	Fortuna	11	9.1
	Soil	Blue Rose	26	7.7
26	Direct	Fortuna	12	66.6
	Soil	Blue Rose	31	64.5
27	Direct	Fortuna	13	100.0
	Soil	Blue Rose	24	81.6
33	Direct	Blue Rose	12	100.0
	Soil	Blue Rose	28	100.0
34	Direct	Blue Rose	13	92.3
	Soil	Rexora	9	66.6
40	Direct	Blue Rose	14	100.0
	Soil	Rexora	11	72.7
41	Direct	Blue Rose	13	84.6
	Soil	Rexora	10	40.0
45	Direct	Blue Rose	47	72.3[a]
	Soil	Rexora	11	90.9
46	Direct	Blue Rose	43	72.1[a]
	Soil	Blue Rose	13	83.8
47	Direct	Early Prolific	15	80.0
	Soil	Blue Rose	13	83.8
52	Direct	Colora	10	50.0[b]
	Soil	Blue Rose	11	100.0
53	Direct	Colora	13	53.8[b]
	Soil	Rexora	10	60.0
Control	Soil	Blue Rose	11	0.0

[a] Shoots were so numerous in the pot that some were missed at time of inoculation.
[b] The upper parts of the sheaths of many of the plants were not in close contact with the stems.

lum was placed on the soil surface. The pots were held in a moist chamber controlled at a temperature of 32° to 33° C. for 48 hours and then removed to the outdoor cage. One pot of well-developed plants was used for each isolate in each series and a pot of nontreated plants was used for a control. It was not possible to use a single rice variety in this test (Table 2).

Where the tissue was inoculated directly, the plants were showing water-soaked areas at the time of removal from the moist chamber and maximum infection was obtained in 7 days. Where the inoculum was placed on the soil surface the plants were showing some infection in 5 days, with maximum infection after about 20 days.

The *Rhizoctonia oryzae* isolates were all highly pathogenic and infection was obtained by both methods of inoculation. On the other hand, cultures of *R. zeae* were highly infectious when the inoculum was placed on the host tissue, but not when placed on the soil surface. The cultures of *R. solani* that were used were mildly to moderately pathogenic, some infection being obtained by both methods of inoculation. The symptoms obtained with all cultures were similar to those observed in the field. Reisolations were made of tissue infected with isolates 20, 21, 22, 25, 26, 27, and 33, and in all cases what appeared to be original organisms were obtained.

Experiment V. Pure cultures of *Rhizoctonia oryzae* (isolate 110) and of *R. solani* (isolates 55, 104, 107, 109) were used in a series of tests conducted in 1937. Isolates 107 and 109 had been obtained from Dr. G. O. Ocfemia and had been isolated from sheath-spot-infected rice plants in the Philippines. Plants were inoculated by placing inoculum behind the leaf sheaths and also by placing it on the soil.

All of the cultures were highly virulent and maximum infection was obtained. All produced essentially the same type of injury, the typical sheath spots characteristic of the disease. The isolates of *Rhizoctonia solani*, however, produced a somewhat larger leaf spot than did the *R. oryzae* isolate, and in the tests in which the inoculation was applied to the soil caused a temporary wilting of the plants. The wilting occurred a very short time after inoculation.

The similarity of the symptoms of sheath spot, as produced by *R. solani*, *R. oryzae*, and *R. zeae* in artificial inoculations, is shown in figure 5, A–D.

Experiment VI. As strains of *Rhizoctonia solani* are known to attack many plants other than rice and are particularly important in "damping off" of vegetable crops, it seemed advisable to try the rice isolates on some of these plants. Tests were conducted on pepper, tomato, eggplant, and bunch beans. Pure cultures were mixed with the soil in wooden flats and in pots and then planted with the seed. Pure cultures of *R. oryzae* (isolates 4, 9, 20, 27, 33), of *R. zeae* (isolate 21), and of *R. solani* (isolates 1, 2, 26, 55, 104, and one that was a mixture of cultures originally isolated from "damped off" pepper plants) were used.

The culture mixture from pepper was the only one of the group that affected germination and subsequent growth of pepper, tomato, and egg-

plant. This completely inhibited the germination of eggplant and reduced by about one-half the germination of tomato and pepper.

Special attention is called to the infection produced on bean plants. Germination was not affected nor were lesions present on any of the stems of plants inoculated with any of the isolates of *Rhizoctonia oryzae* or *R. zeae.* On the other hand, all of the *Rhizoctonia solani* isolates affected the

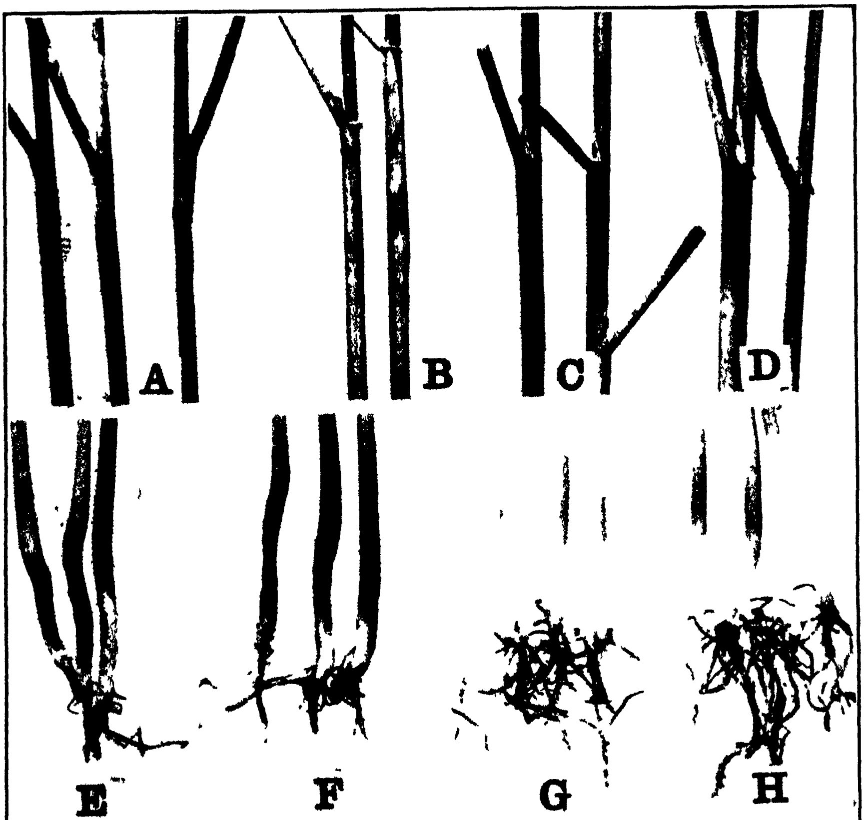

FIG. 5. A–D. Sheath spots on rice produced by four different isolates of Rhizoctonia. A. *R. solani* (isolate 104). B. *R. solani* (isolate 55). C. *R. oryzae* (isolate 27). D. *R. zeae* (isolate 21). E–H. Injuries produced on young bean plants following inoculation with *Rhizoctonia solani* and *R. oryzae.* E. *R. solani* (isolate 2). F. *R. solani* (isolate 104). G. *R. oryzae* (isolate 5). H. Control.

bean plants very seriously. Germination was materially reduced and lesions were present on most of the young plants that did develop. These differences emphasize the distinction between *R. solani* and *R. oryzae.* Representative seedlings of this experiment are shown in figure 5, E–H.

Experiment VII. Two tests were conducted to determine whether or not any of the *Rhizoctonia* isolates would reduce the germination of rice seed or cause a seedling blight. Four-gallon pots, filled with soil and then steam-sterilized were inoculated with pure cultures of *R. solani, R. zeae,* and *R. oryzae* (the same isolates used in Experiment VI). Two pots were used

with each isolate in the first test and 3 in the second. In each pot were planted 100 Blue Rose rice seeds previously sterilized 4 minutes in a 1–1,000 corrosive sublimate solution in 50 per cent alcohol. The tests were started on February 17 and March 5, 1937, respectively.

Two of the *Rhizoctonia solani* isolates (isolates 1 and the mixture from "damped-off" pepper plants) definitely reduced germination. In the pots inoculated with these cultures there were about one-third as many plants as in the controls. There was also a small amount of seedling blight in these pots.

The other isolates had no effect on germination. Under the conditions of the experiment *Rhizoctonia oryzae* was not a factor in seedling blight.

RELATION OF TEMPERATURE TO FUNGUS GROWTH

Preliminary studies on the temperature relations of *Rhizoctonia solani* and *R. oryzae* showed that the optimum for growth of *R. oryzae* was close to 32° C., while for the isolates of *R. solani* it was nearer 28° C. The following experiment was designed to check these findings more closely.

Pure cultures of *Rhizoctonia solani* (isolates 1, 2, 26, 55, 104), *R. zeae* (isolate 21), and *R. oryzae* (isolates 4, 9, 20, 27, 33) were grown on bean-pod agar. When the cultures filled the Petri dishes, discs 5 mm. in diameter were cut from the margins of the colonies and transferred to freshly poured potato-dextrose agar plates and then incubated in triplicate at the following temperatures: 10, 15, 20, 24, 28, 32, and 35° C. The diameters of the resulting colonies were measured daily. Most isolates at optimum temperature for growth covered the plates by the end of 48 hours. The growth rates for *R. oryzae* and *R. zeae* were essentially the same. There was very slight growth at 10° C., good growth at 24° to 35° C., with an optimum close to 32°. There was only a very slight drop in the growth rate at 35° C. On the other hand, *R. solani* developed better at somewhat lower temperatures. There was slight growth at 10° C., good growth at 24° to 32° C., with an indicated optimum between 28° and 32° C. and a very sharp drop in growth at 35°. The strains of *R. solani* with small sclerotia (isolates 1 and 2) grew somewhat slower than did the strains with large sclerotia (isolates 26, 55, and 104). This experiment was repeated with essentially the same results. The growth curves of three isolates, 1, 4, and 104, representative of the three species of *Rhizoctonia*, are shown graphically in figure 6.

DISCUSSION

In the Eastern Hemisphere a sheath spot of rice, which seems to be very common and frequently serious, has been known for some years. In some of the Oriental countries this disease is said to be caused by *Rhizoctonia solani* and in others by *Hypochnus sasakii*. From a study of the literature, however, it would seem that the Oriental investigators have been working with a single organism, a strain of *R. solani* having large sclerotia.

A sheath spot of rice also occurs in Louisiana. While it has symptoms

indistinguishable from the Oriental disease, it seems to be of minor importance. The fungus which is responsible for most of the sheath spotting in Louisiana is also a *Rhizoctonia,* but one that is definitely different from the *R. solani* reported from the Orient. This fungus seems to be different enough to be regarded as a distinct species and in this article is described as new under the name *R. oryzae.* Strains of *R. solani* and *R. zeae* that are capable of producing sheath spots have also been found on rice in Louisiana, but these occur so infrequently that they seem to be of little importance.

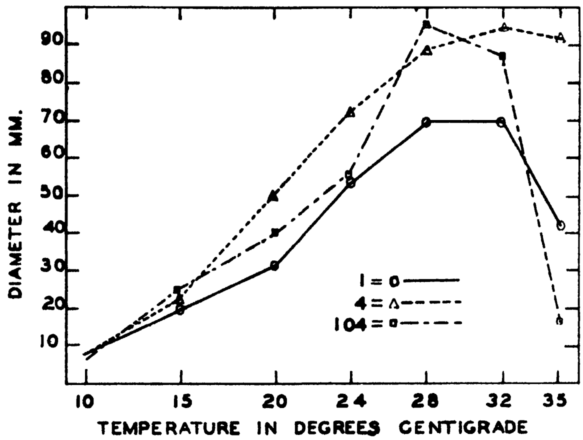

FIG. 6. Graph showing the average diameters of two isolates of *Rhizoctonia solani* (1 and 104) and one isolate of *R. oryzae* (4) on potato dextrose agar at various constant temperatures at the end of 50 hours.

These conclusions are based on a study of many hundreds of collections and isolations from rice and inoculation tests in which cultures from China and the Philippine Islands have been included.

Rhizoctonia oryzae is entirely distinct from the *Rhizoctonia* strains ordinarily found in Louisiana soils, the ones that attack a wide range of vegetable and field crops and cause such troubles as "damping off." It has not been possible to obtain infection on such crops as pepper, eggplant, tomato, and beans with this fungus.

Inoculation tests with pure cultures of *Trichoderma,* the fungus that Tullis has reported as the causal organism of the rice sheath spot, have been negative. While species of *Trichoderma* are common in Louisiana soils, present evidence indicates none of them is responsible for the sheath spot.

SUMMARY

The symptoms and causal organism of a sheat spot of rice in Louisiana are described.

Evidence is presented to indicate that the disease is caused by a hitherto-undescribed species of *Rhizoctonia.*

The organism is given the name ***Rhizoctonia oryzae,*** sp. nov.

LOUISIANA AGRICULTURAL EXPERIMENT STATION
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THE VALUE OF NEW COPPER SPRAYS AS FUNGICIDES FOR THE CONTROL OF APPLE BLOTCH, CHERRY LEAF SPOT, AND APPLE SCAB—1937

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(Accepted for publication December 20, 1937)

No spray is so universally recommended and at the same time condemned as our old stand-by, Bordeaux mixture. In spite of the fact that it has been the subject of intensive study ever since its introduction as a spray in 1882, little has been accomplished in eliminating the evils so commonly associated with its use. Its effect upon the normal physiological functions of plants is a matter of common knowledge. Likewise, we understand quite well the conditions under which it causes chemical burning, as well as desiccation resulting from excessive water loss.

Actuated by the knowledge of the shortcomings of Bordeaux, many workers have been busy in the last few years trying to find a suitable substitute for it among the so-called "insoluble" copper compounds.

The introduction of so many new materials recommended for such a variety of purposes has resulted in the usual confusion and contradictions. The aftermath of such a situation is the necessity for carefully done comparative tests under a wide variety of conditions and environments. Such tests over a period of years usually produce the necessary "testimonials" of any material's performance.

Literature reviews on this general subject have been given by Roberts[1] and by Marsh.[2] Since their papers were published, additional work has appeared in PHYTOPATHOLOGY and in bulletins of the Arkansas, Michigan, and Ohio Agricultural Experiment Stations and of the Delaware State Board of Agriculture.

We realize that the data herein reported represent only one season's results, but we feel completely justified in presenting them in that the season was ideal for the comparison of spray materials. The severity of the diseases under consideration bears out this contention.

MATERIALS AND METHODS

The trade name of the materials considered in this year's program, the active ingredients of each, and the companies that supplied them are as follows:

Name	*Principal active ingredients*	*Furnished by*
Bordeaux mixture	Copper sulphate	Purchased
Oxo Bordeaux	Copper sulphate	Ansbacher-Siegle Corp.
Basi Cop	Basic copper sulphate	Sherwin-Williams Co.
Coposil	Copper calcium-copper zinc silicate	Calif. Spray Chem. Co.

[1] Roberts, J. W. Recent developments in fungicides: spray materials. Bot. Rev. 2: 586–600. 1936.

[2] Marsh, R. W. Some recent American work on the copper fungicides. Sci. Hort. [Wye, Kent, Eng.] 5: 60–66. 1937.

Name	*Principal active ingredients*	*Furnished by*
Copper phosphate	Copper phosphate	Monsanto Chemical Co.
Cupro-K	Copper oxy chloride	Rohm and Haas Co.
Cuprocide 54	Cuprous oxide	Rohm and Haas Co.
Z-O	Copper ammonium silicate	Nichols Copper Co.
Bordeaux "34"	Basic copper sulphate	General Chemical Co.
Copper Hydro 40	Copper hydroxide	Chipman Chemical Co.
Special Copper	Basic copper sulphate	Niagara Sprayer & Chemical Co.
66a	an organic sodium sulphonate plus copper sulphate	National Aniline & Chemical Co
Liquid lime sulphur	various forms of sulphur	Purchased

Each material was used as recommended by the company that furnished it, unless otherwise stated. All sprays were applied with power machines at a pressure of 400 to 600 pounds. Notes were taken every week or 10 days throughout the season concerning the general tree and fruit condition.

The season may best be described by saying that it was ideal for the development of apple scab, apple blotch, cherry leaf spot, and spray injury caused by water-soluble chemicals. In addition to frequent showers, there were numerous misty days, as well as heavy dews at night. The actual temperature and precipitation for the vicinity of Urbana are shown in figure 1, but the graph does not convey an accurate picture of climatological conditions unless the heavy, muggy weather be taken into consideration.

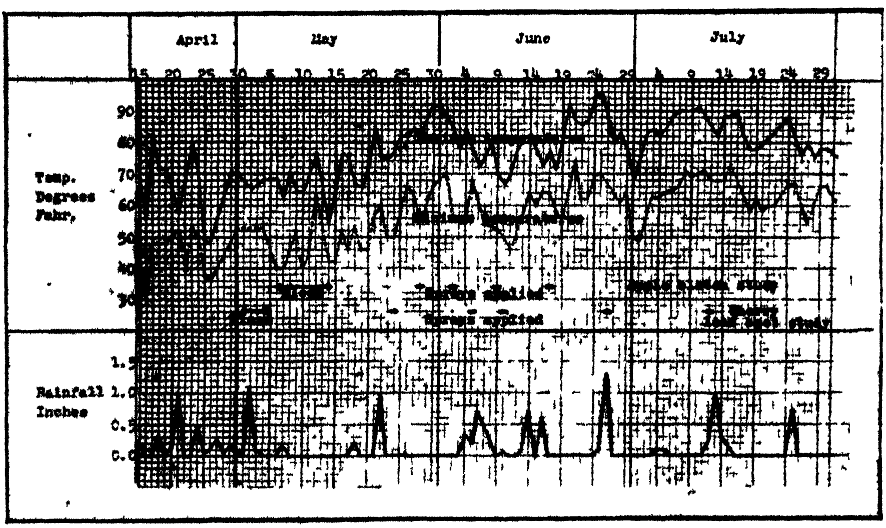

FIG. 1. Record of temperature and rainfall, Urbana, Illinois, April–July, 1937.

THE RELATION OF NEW COPPER MATERIALS TO APPLE BLOTCH SPRAYS

In this experiment the objectives were to evaluate the various copper materials from the standpoint of blotch (*Phyllosticta solitaria*) control, spray injury, and compatibility with fixed nicotine (Black Leaf 155), which is currently being recommended for codling-moth control on early apple varieties in Illinois.

The experiments were conducted in the orchards of Fred Heaton (southern Illinois, New Burnside) and George Livingston (Savoy, 3 miles south of Urbana).

Aside from a little injury to the leaves caused by Cuprocide 54, no significant results were obtained from the plots in southern Illinois. In the nonsprayed checks there was less than 1 per cent of blotch or codling-moth injury to the fruit and, accordingly, the details of this experiment will not be given.

All trees used in the Savoy (Livingston orchard) apple-blotch experiment were of the Duchess variety and were especially selected for the presence of numerous viable blotch cankers on branches and twigs. The actual sprays applied, with dates of application, are given in the following spray schedule.

TABLE 1.—*Schedule of sprays applied to Duchess apples in the Livingston Orchard, Savoy, Illinois (Spray schedule No. 1)*

Plot No.	Materials used[a]	1st Cover 5/27/37	2d Cover 6/2/37	3d Cover 6/9/37	4th Cover 6/17/37	5th Cover 7/6/37	6th Cover 7/16/37
1	Black Leaf 155	—	—	6	6	6	6
		No fungicide applied after calyx					
2	Copper sulphate	4	4	4	4	—	—
	Lime	6	6	6	6	—	—
	Black Leaf 155	—	—	6	6	6	6
3	Bordeaux 34	1½	1½	2	2	—	—
	Zinc sulphate	½	½	¾	¾	—	—
	Lime	½	½	¾	¾	—	—
	Black Leaf 155	—	—	6	6	6	6
4	Oxo Bordeaux	6	6	4	4	—	—
	Black Leaf 155	—	—	6	6	6	6
5	Basi Cop	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
6	Copocil	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
7	Copper phosphate[b]	2	2	3	3	—	—
	Lime	4	4	5	5	—	—
	Bentonite	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
8	Cupro-K	3	3	4	4	—	—
	Black Leaf 155	—	—	6	6	6	6
9	Cuprocide 54	2	2	2	2	—	—
	Black Leaf 155	—	—	6	6	6	6
10	Copper Zeolite	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
11	66a	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
12	Copper Hydro 40	2	2	4	4	—	—
	Black Leaf 155	—	—	6	6	6	6
13	Niagara Copper	2	2	3	3	—	—
	Black Leaf 155	—	—	6	6	6	6
14	Check	No sprays applied after calyx					

[a] All trees in the experiment received one early scab spray of flotation sulphur, as well as lead arsenate (3), lime (3), and flotation (16), as a calyx application. All materials expressed as pounds in 100 gallons of water.

[b] Used at ½ recommended strength by mistake.

The first two cover sprays were applied at strengths recommended for apple-scab control. This was done, since apple-scab studies also were being conducted in this orchard, and application of materials of the same strength to both experiments greatly facilitated the task of applying the sprays. The third and fourth cover sprays were as recommended for blotch by the companies that furnished the materials, unless otherwise stated.

The records include all the drops and picked fruit from 2 trees in each plot. Since the drops in no instance appeared related to spray injury, but, instead, to the lack of codling-moth control, and, since the differences between individual trees in a given plot were insignificant, the results (Table 1) are listed in percentages and based upon the total fruit examined in each plot.

It may be observed (Table 2) that blotch was extremely severe in the

TABLE 2.—*Value of insoluble copper sprays as possible substitutes of Bordeaux mixture for the control of apple blotch. Experiment at Savoy, Illinois, on the variety Duchess, 1937*

Material used[a]	Per cent blotch on		Spray injury to	
	Fruit	Petioles[b]	Fruit[c]	Leaves
Black Leaf 155 only	92.0	95.0 50% leaf fall	0.0	None
Bordeaux Black Leaf 155	0.7	Trace	3.0	Severe
Bordeaux 34 Zinc sulphate and lime Black Leaf 155	2.4	3.0	0.7	Trace
Oxo Bordeaux Black Leaf 155	1.6	5.0	0.4	None
Basi Cop Black Leaf 155	1.0	Trace	41.0	Moderate
Copocil Black Leaf 155	4.5	6.0	4.0	Light
Copper phosphate Lime and bentonite Black Leaf 155	3.1	15.0	Trace	Trace
Cupro-K Black Leaf 155	2.8	5.0	2.0[d]	Trace
Cuprocide 54 Black Leaf 155	2.5	Trace	49.0	Severe
Copper Zeolite Black Leaf 155	1.2	5.0	0.9	None
66a Black Leaf 155	17.0	30.0 15% leaf fall	Trace	None
Copper Hydro 40 Black Leaf 155	1.9	5.0	Trace	Trace
Niagara Copper Black Leaf 155	1.4	5.0	4.0	Moderate
Check—Unsprayed	97.0	95.0 60% leaf fall	0.0	None

[a] See Spray Schedule No. 1 for details of applications.
[b] Estimated on October 6—about 6 weeks after harvest.
[c] Only the injury of commercial importance was recorded.
[d] Nearly all fruit showed a little specking, but it was of no commercial concern.

plots (1 and 14) that did not receive a fungicide for its control; in fact, it was the most severe that it has been in Illinois for several seasons. Fruit from all nonsprayed Duchess trees in the general vicinity of Urbana was almost worthless from a commercial point of view. Under such conditions it was indeed gratifying to obtain such excellent control with so many of the insoluble copper compounds. Of all the materials tested, 66a (Plot 11) was the only one that did not give satisfactory control of blotch. In this plot the infected fruit, leaves, and petioles were badly spotted, while in all other sprayed plots the infected organs showed only a few isolated lesions each.

In all the experiments herein discussed, copper phosphate was used by mistake at one-half the concentrations recommended; but, in spite of this fact, it gave good control of blotch on the fruit and leaves. The petioles, however, were quite badly diseased. Further tests of this spray would certainly be justified.

Several of the materials as now recommended produced injury on both the fruit and foliage. Injury of commercial concern was caused to both fruit and foliage by Cuprocide 54, Basi Cop, Bordeaux mixture, the special copper from Niagara Sprayer and Chemical Company, and Coposil. Cupro-K was about on the border line of safety in this test, since a large portion of fruit showed a few typical copper specks but not enough of them to reduce the grade or marketability of the apples. It caused no noticeable injury to the leaves.

The data concerning the influence of the various copper compounds on the control of codling moth by Black Leaf 155 are not considered significant because of the light infestation by worms and the variation of infestation between trees in any given plot.

Thus, both from the standpoint of spray injury and apple-blotch control, the copper compounds listed below were found to be entirely satisfactory for use in the Illinois apple-blotch spray schedule this year. The materials and concentrations used per 100 gallons of water were Bordeaux 34, 2 pounds, with zinc sulphate, ¾ pound and lime ¾ pound; Oxo Bordeaux, 6 pounds; Copper Zeolite, 3 pounds; Copper Hydro 40, 3 pounds; Cupro-K, 3 pounds; and possibly copper phosphate, 4 pounds, with lime, 6 to 8 pounds and bentonite, 4 pounds.

STUDIES ON THE CONTROL OF CHERRY LEAF SPOT, 1937, AT UNIVERSITY FARM, URBANA, ILLINOIS

Because of diseases, insect pests, and climatic conditions, very few cherries are grown commercially in Illinois. Accordingly, any comprehensive studies on the control of cherry leaf spot (*Coccomyces hiemalis*) would hardly be justified, except that such a study supplies additional evidence in the general process of evaluating fungicides. It is with this in mind that the following experiment was undertaken.

The plots were located on the university farm at Urbana, Illinois, and were composed of Montmorency, Early Richmond, and Dyehouse, varieties of sour

cherry. Each week after the first application was made, records of the general tree condition and cherry leaf spot were made. Because of irregularity in size and general condition of individual trees, no records were secured on the effect of the various sprays on fruit size.

Leaf spot appeared a little earlier this year than usual and, as a result, was established in all plots, on the leaves within three feet of the ground, before any sprays were applied. In some of the plots the materials definitely burned out the spots. In others the fungus remained viable or would spread slowly during the spraying season and very rapidly after the last spray was applied. The applications were made on May 25, June 5, 10, and 26, and on July 12. Only a few plots were sprayed on June 5, when a driving rain interrupted the procedure. The rest of the plots were sprayed later in the day, between showers. In several instances the materials were not thoroughly dry before further rains fell (Fig. 1). Because of this condition all plots were sprayed again on June 10, which was as soon as other work would permit. All other sprays were timed at about 2-week intervals.

Although records were taken of the actual percentage of leaf fall in each plot and on the percentage of leaves remaining on the trees that were diseased, these data will not be presented because in many cases they give the wrong impression as to the actual plot condition. This is due largely to the fact that it was often difficult to differentiate clearly between defoliation resulting from spray injury and that caused by cherry leaf spot.

In the plot ratings here presented, each material is rated on the basis of 1 to 10; 1 indicating that the plot was in very good condition throughout the

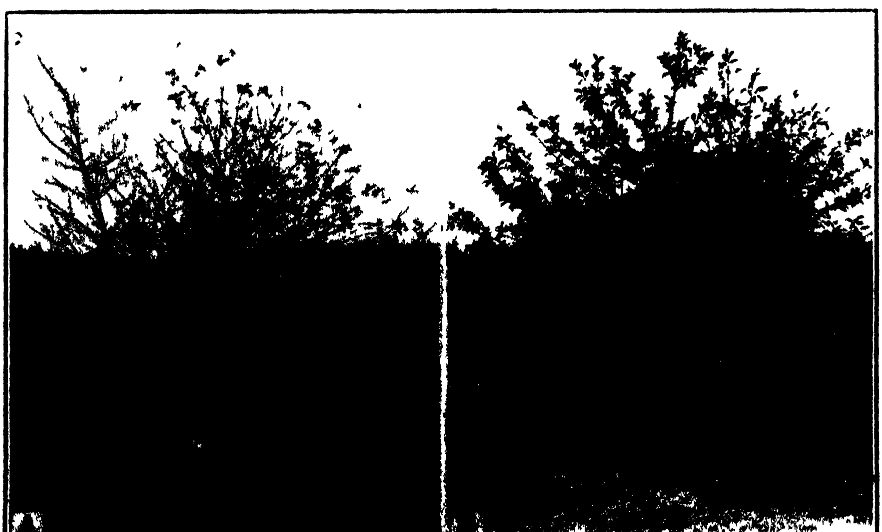

FIG. 2. A. Nonsprayed check. Most of the leaves were off this three two weeks before harvest. This was a very bad leaf-spot year in Illinois. B. Tree sprayed with Bordeaux 34, zinc sulphate, and lime at the rate of 1½ lbs., ½ lb., ½ lb. to 100 gallons of water. This was one of the most successful sprays in the experiment. It gave almost perfect control of leaf spot without spray injury.

season, whereas 10 relates to the condition of the nonsprayed checks, which were almost defoliated two weeks before harvest.

DISCUSSION OF CHERRY SPRAYING RESULTS

Based on the results of this year's cherry leaf-spot studies, conducted under conditions especially conducive to spray injury and the development of cherry leaf spot, it appears that some of the new copper fungicides have definite promise as substitutes for Bordeaux mixture. The 3 sprays that controlled cherry leaf spot without causing commercially significant injury

CHERRY LEAF SPOT—GENERAL PLOT RATINGS, 1937

Plot rating	Comments and summary
10	*Nonsprayed Checks* (Fig. 2, A) Trees in very bad condition. They were defoliated within the first week of June and remained so throughout the season, except for a few leaves that continued to develop on the tips of the branches. Even these leaves became diseased soon after they appeared. About 90 per cent of leaves off.
1	*Bordeaux 34,* 1½ lbs.,[a] with *zinc sulfate,* ½ lb. and *lime,* ½ lb. (Fig. 2, B) [a] Weights given are based on 100 gallons of spray. This was one of the best looking plots in the experiment. Except for a little browning on the under surface of the leaves, there was no injury. Early leaf spot infections burned out. Excellent leaf color. About 2 per cent of the leaves fell during season.
2	*Niagara Special Copper,* 2 lbs. Early in the season this plot showed considerable spray injury to the leaves. It has given excellent control of leaf spot throughout the season. The plot appeared in good condition late in the season. The leaves showed considerable browning on their under surface. Eight to 10 per cent of the leaves fell because of early season injury. This material has promise, but must be made safe before it is used for leaf spot control.
2	*Copper Hydro 40,* 2 lbs. This plot showed a little injury early in the season, but at no time was it of any great commercial concern. Underside of leaves showed considerable browning. Leaf color good. Excellent control of leaf spot throughout season. Material should be made a little safer before it is recommended to growers. About 5 per cent defoliation because of early season injury.
8	*66a,* 2 lbs. No spray injury to leaves, but a dark specking of the fruit associated with the lenticels was observed. At concentrations used, this product was of no value for leaf spot control. Leaves started falling before harvest and soon thereafter over 80 per cent were off. Late in the season, plot was about 95 per cent defoliated, with remaining leaves infected.
4	*Copper Zeolite,* 2 lbs. Only evident spray injury to leaves was a moderate browning of dorsal surface. Leaf color good. Some black specks on the fruit associated with lenticels. Injury as a whole of no importance. Plots sprayed with this material looked very good until shortly after harvest. After the last spray application was made, leaf spot began to defoliate, eventually causing about 60 per cent of the leaves to fall. Most of those remaining were diseased. Either this material does not adhere to the leaves well or else concentration was too low to be effective.
6	*Cuprocide 54,* 2 lbs. From the start this plot showed severe spray injury. All leaves off color with under surfaces badly browned. Good control of leaf spot. Between 40 per cent and 50 per cent leaf fall due, apparently, to spray injury.

CHERRY LEAF SPOT—GENERAL PLOT RATINGS, 1937—(*Continued*)

Plot rating	Comments and summary
	Cupro-K, 2 lbs. (Fig. 3, A) A little spray injury early in the season. Leaves show some browning on underside. Injury of doubtful commercial significance. Excellent control of cherry leaf spot. Early leaf spot all burned out. Leaf color good. About 3 per cent defoliation early in season as result of injury.
2	*Copper Phosphate*, 2 lbs., with *lime*, 4 lbs. and *bentonite*, 2 lbs. Used at one-half recommended strength. A little browning on underside of leaves only evidence of spray injury. Leaf color excellent; deeper green than in any other plot. About 15 per cent of leaves fell because of leaf-spot infections. At strength used, material seems unable to stop the disease once it is established.
2	*Coposil*, 2 lbs. This plot showed considerable spray injury, early in the season, that resulted in about 10 per cent leaf fall. Remaining leaves showed some browning; slightly off color. Leaf-spot control good; early spots burned out. Coposil needs to be made safe for use on cherries, but has very definite promise.
2	*Basi Cop*, 2 lbs. Moderate early-season spray injury, resulting in loss of about 10 per cent of leaves. Remaining leaves showed browning on underside and were definitely off color. Good control of leaf spot; early spots burned out. Like some of the other materials, Basi Cop has definite promise if made safe as a fungicide.
3	*Oxo Bordeaux*, 3 lbs. Since this product was used at only half strength, the results are of little value in evaluating it. No injury, except a little browning on underside of leaf, was evident throughout the season. Disease spread slowly until after last spray was applied and then spread quite rapidly. At end of season about 35 per cent of leaves were off and most of those remaining showed infections. Further tests of this product at 6 lbs. per 100 gallons seem justified.
3	*Bordeaux mixture*, 4–6–100 This plot, unlike some of the other copper plots, showed no spray injury until about one month after harvest. From that time on, the injury became increasingly worse until, by the middle of September, over 70 per cent of the leaves were off. Excellent control of leaf spot was secured. Leaves were browned on underside and off color after injury began to appear.
1	*Liquid Lime Sulphur*, 2 gallons (Fig. 3, B) This was another of the best plots in the experiment. No injury was evident throughout the season. Very little browning on underside of leaves, which had excellent color all season long. A little leaf spot was evident in the plots about one month after harvest. It continued to develop until about 10 per cent of the leaves fell and at least 25 per cent more were infected.

were Bordeaux 34, used with zinc sulphate and lime; Cupro-K; and liquid lime sulphur.

Other materials that gave very good control of leaf spot, but must be made safe for use on cherries, were Copper Hydro 40; Coposil; Basi Cop, the special copper from the Niagara Sprayer and Chemical Company; and Cuprocide 54.

Since both Oxo Bordeaux and copper phosphate were used at weaker dilutions than recommended, further tests are necessary before commenting on them.

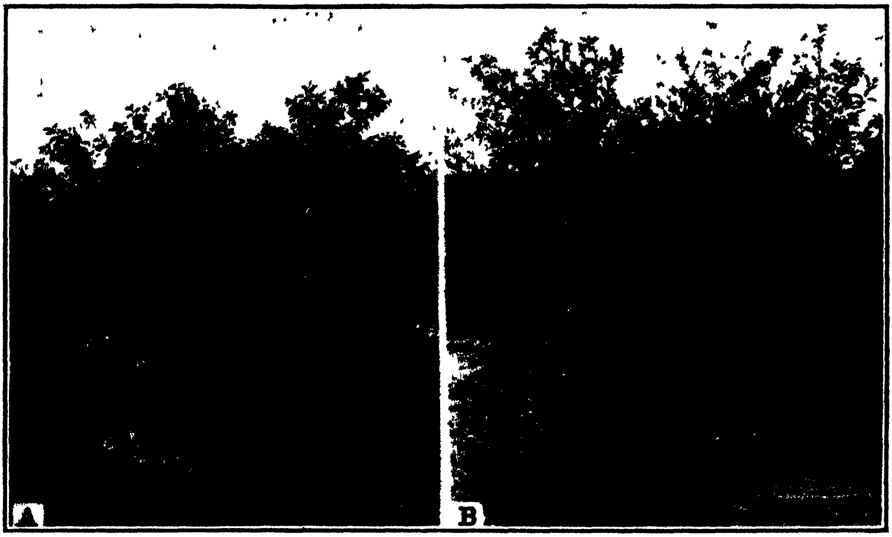

FIG. 3. A. Cupro-K, 2 lbs. per 100 gallons, was the spray used on this tree. It gave excellent control of leaf spot with only a little injury early in the season. The injury was of no commercial concern. B. Tree sprayed with liquid lime sulphur at the rate of 2 gallons per 100 gallons of water. This was a very good plot until about 4 to 5 weeks before frost, when leaf spot began to cause leaf fall. About 10 per cent of the leaves have fallen from this tree.

COMMENTS CONCERNING COPPER SPRAYS FOR APPLE SCAB CONTROL

Since our results concerning the use of the various copper sprays for the control of apple scab (*Venturia inaequalis*) will appear in the 1937 "Transactions of the Illinois State Horticultural Society," it does not seem desirable to give more than a brief summary of the findings at this time.

All of the new sprays tested, except 66a, gave satisfactory control of apple scab, but the copper sprays are not advised in the apple-scab schedule because of the injury they are likely to produce.

Certain sulphur sprays may be used effectively up to and including the first cover spray (1 week after calyx), without injury, even though oil is to be used in the third cover. Weather conditions, which occasionally justify second cover scab sprays, are essentially the same as those most likely to produce copper burn. Under experimental and commercial conditions several instances of severe fruit russet from one application of copper-spray materials in either the first or second cover sprays or from an application in each of them have come to our attention this season. This condition was observed on Jonathan, Winesap, Ben Davis, and Delicious. In another instance where Delicious was sprayed in the first and second covers with various copper sprays, no injury resulted from many of the materials used.

DISCUSSION

Although the results herein reported represent only one year's experiments, we present them, nevertheless, with the hope of contributing to the

present efforts of evaluating the various possible substitutes for Bordeaux mixture. Presentation of a single year's data is further justified by the fact that the 1937 season, in Illinois, was ideal for the development of apple blotch in the vicinity of Urbana, apple scab, and cherry leaf spot, and of spray injury from water-soluble chemicals.

Apple Blotch

All materials, except 66a, gave satisfactory control of apple blotch on fruit and leaves. Through a mistake, copper phosphate, lime, and bentonite were used in all of this year's tests at one-half recommended strength. In spite of this fact, there was good control of apple blotch on fruit and leaves, but, along with 66a, these materials did not control the disease satisfactorily on the petioles.

Both from the standpoint of blotch control and spray injury, the following materials were satisfactory this year (concentrations in 100 gallons): Bordeaux 34, 2 lbs., with zinc sulphate, $\frac{3}{4}$ lb., and lime, $\frac{3}{4}$ lb.; Oxo Bordeaux, 6 lbs.; Copper Zeolite, 3 lbs.; Copper Hydro 40, 3 lbs. Cupro-K is recommended for test at 3 lbs., since, at 4 lbs., it was at the border line of safety, causing a little specking of the fruit.

Copper phosphate, 4 lbs., lime, 6 to 8 lbs., and bentonite, 4 lbs. seem to justify further consideration as a blotch spray, since, used at one-half the above concentration, it controlled all phases of blotch, except on the petioles, without any sign of injury to the tree or fruit.

The other materials that controlled blotch, but caused injury of commercial importance to either the fruit or leaves or both, are Cuprocide 54, Basi Cop, Bordeaux mixture, the special copper from the Niagara Sprayer and Chemical Company, and Coposil.

No data of significance were obtained this season on the combatability of the various materials tested with fixed nicotine, insofar as codling-moth control was concerned.

Cherry Leaf Spot

Most of the companies that furnished materials recommended smaller concentrations for the control of cherry leaf spot than for apple blotch. This, together with the fact that applications were made at greater intervals than in the blotch study, has caused some materials that gave excellent control of blotch to fall down in the control of cherry leaf spot. Whether or not their failure to control was a function of poor weathering or the dilution of the material was not determined.

In this study both copper phosphate and Oxo Bordeaux were used at one-half the recommended strength. Both sprays so used, as well as Copper Zeolite, failed to control leaf spot after the last sprays were applied, although they held the disease in check quite well until after harvest. 66a was of no value in cherry leaf-spot control. All other materials used controlled leaf spot but many of them caused rather serious spray injury.

From the standpoint of cherry leaf-spot control and spray injury, the following materials were satisfactory this year as recommended and used (concentrations in 100 gallons): Bordeaux 34, 1½ lbs., zinc sulphate, ½ lb., and lime, ½ lb.; Cupro-K, 2 lbs.; liquid lime sulphur, 2 gallons.

Other fungicides that have definite promise as cherry leaf-spot sprays, but that must be made safe before they are used, are Copper Hydro 40, Coposil, Basi Cop, and the special copper from the Niagara Sprayer and Chemical Company.

Apple Scab

It is doubtful if there is any place in the Illinois apple scab schedule for the use of copper fungicides, because of the injury they cause under Illinois climatic conditions. All of the materials tested, except 66a, gave satisfactory control of scab.

RÉSUMÉ

It seems safe to say that some of the new copper fungicides have definite promise as possible substitutes for Bordeaux mixture. Not many of them, however, are safe for fruit-tree spraying as now recommended. Some of the products that caused spray injury could undoubtedly be rendered safe without affecting materially their fungicidal values. A few of the products that appear to be quite safe as now recommended need further study to determine satisfactory concentrations for use against the various diseases.

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THE SPREAD OF ONION MILDEW BY WIND-BORNE CONIDIA OF PERONOSPORA DESTRUCTOR

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Since Cook's (2) discovery of the mycelium of *Peronospora destructor* within the seed of onion and Leach's (5) demonstration of seed transmission of the downy mildew of beet, the writer (12) has obtained circumstantial, though not positive, evidence of seed transmission in onions, which seemed to warrant extensive tests of seed treatment by hot water as a means of gaining further corroboratory evidence of Cook's theory.[1] Since the field evidence indicated that seed transmission might be comparatively rare (1 seed out of many thousands), the field tests with hot-water-treated seed have been extensive rather than intensive, involving treatment of nearly a ton of seed obtained from over 100 onion growers during the past 3 years.

During this period, mildew occurred on many of the farms where these tests were being conducted. On a few of them it appeared first in the plots sown with nontreated seed; on a very few, however, it appeared first in the plots sown with treated seed. In most cases no striking difference in time of appearance or severity of mildew could be observed. During the past sea-

[1] The treatments employed after laboratory tests of many samples, consisted of immersion in hot water at 51° C. for 22 to 25 minutes.

son, moreover, which was the only one of the 3 in which mildew reached epiphytotic proportions,[2] the behavior of the disease in certain experimental plots clearly indicated that there were some sources of primary inoculum other than that being investigated and that were fully as early and probably more important. These sources include the Egyptian or topset onions grown in backyard gardens, together with their related potato or multiplier onions, and the bulbs carried through the winter in storage by many growers who grow their own seed.

It has been known since the work of Murphy and M'Kay (9) and of Katterfeld (4) that *Peronospora destructor* lives over as perennial mycelium in such bulbs, but the importance of these sources of primary inoculum has been discounted in America for 3 reasons: The topset onions are not believed to be grown commonly enough to be a factor; they seldom if ever are grown close enough to commercial fields to be a menace; and the conidia of *P. destructor,* in common with other members of the Peronosporaceae, are thin-walled, delicate, easily desiccated, and are, therefore, considered incapable of surviving transportation by air to any distance. The data presented here are in disproof of these 3 suppositions.

FIRST CLUE TO AERIAL DISSEMINATION

The most striking piece of field evidence pointing to the importance of seed bulbs and topsets as sources of primary inoculum was obtained in a muckland valley devoted largely to onion and celery culture in Wayne County, where experimental plots were laid out comparing hot water and hot acetic acid treatments of suspected commercial seed with home-grown, mildew-free seed.

The spring and early summer of 1937 were unusually wet and foggy and very favorable for the development of mildew, which was first found on one or two garden plantings of topsets in Wayne County in mid-May. By the last week in June, mildew was found in all of the seed-treatment plots in the experimental acre referred to, but its occurrence was hardly what would have been expected had it been seed-borne. Individual primary infections were rather evenly scattered over the entire field, strongly indicating a distant source of inoculum. The plots from home-grown seed had as much disease as did those from treated commercial seed. The checks had no more, and, although the hot acetic acid plots showed fewer lesions, this could be accounted for by the fact that the seed treatment had greatly reduced the stand so that there were fewer leaves to the acre to arrest the falling conidia as they drifted through the air.[3] The counts and stands are given in table 1.

That the primary conidia did come through the air in this case was soon shown to be likely. By making counts of the number of blighted leaves per

[2] In 1937 the Northeastern States suffered one of the worst outbreaks of mildew on record, due largely to a very wet May and June.

[3] The treatment consisted of immersion in hot water at 50° C. for 25 minutes. The .5 per cent acetic acid bath was at the same temperature for 20 minutes.

TABLE 1. *Failure of onion mildew control by seed treatment as indicated by the distribution of primary infections on various plots. July 8, 1937*

Source and treatment	Stand of onions per 100 ft. of row	Lesions of mildew per 100 ft. of row
Home-grown Y.G. (disease-free)	1200	1.62
Brig. Y.G. (Check) (suspected)	1408	1.25
Brig. Y.G. (Hot water)	1361	1.60
Brig. Y.G. (Hot acetic)	735	.38

100 feet of row in all the fields in this valley, to the northwest, and to the south of the experimental plots, it was found that there was progressively more mildew as one moved from south to northwest. The lesions appeared to be of the same age until one reached the northwest corner of the valley, where, obviously, the source of inoculum was located. This area consisted

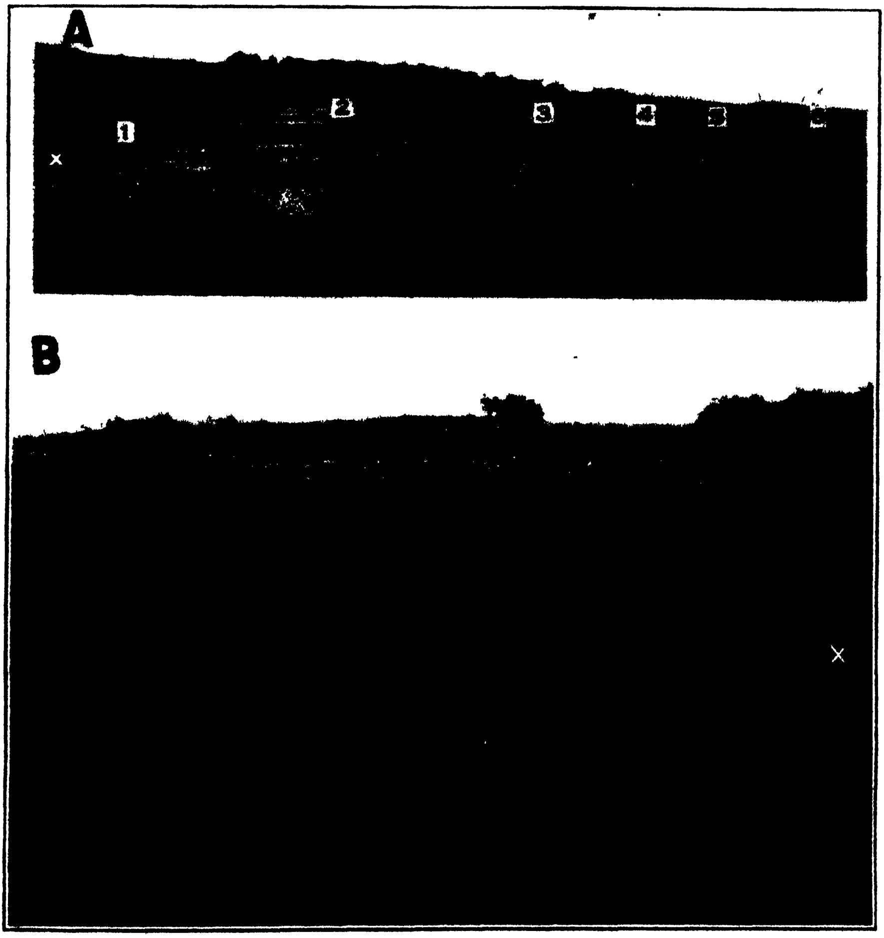

FIG. 1. A. General view of valley where onion-mildew seed-treatment tests were conducted, looking S.E. Source of primary infection (X) left center. Location of fields referred to in table 2, indicated by numbers 1 to 6. B. Near view of source of primary infection in onions being grown for seed (shown at X in A).

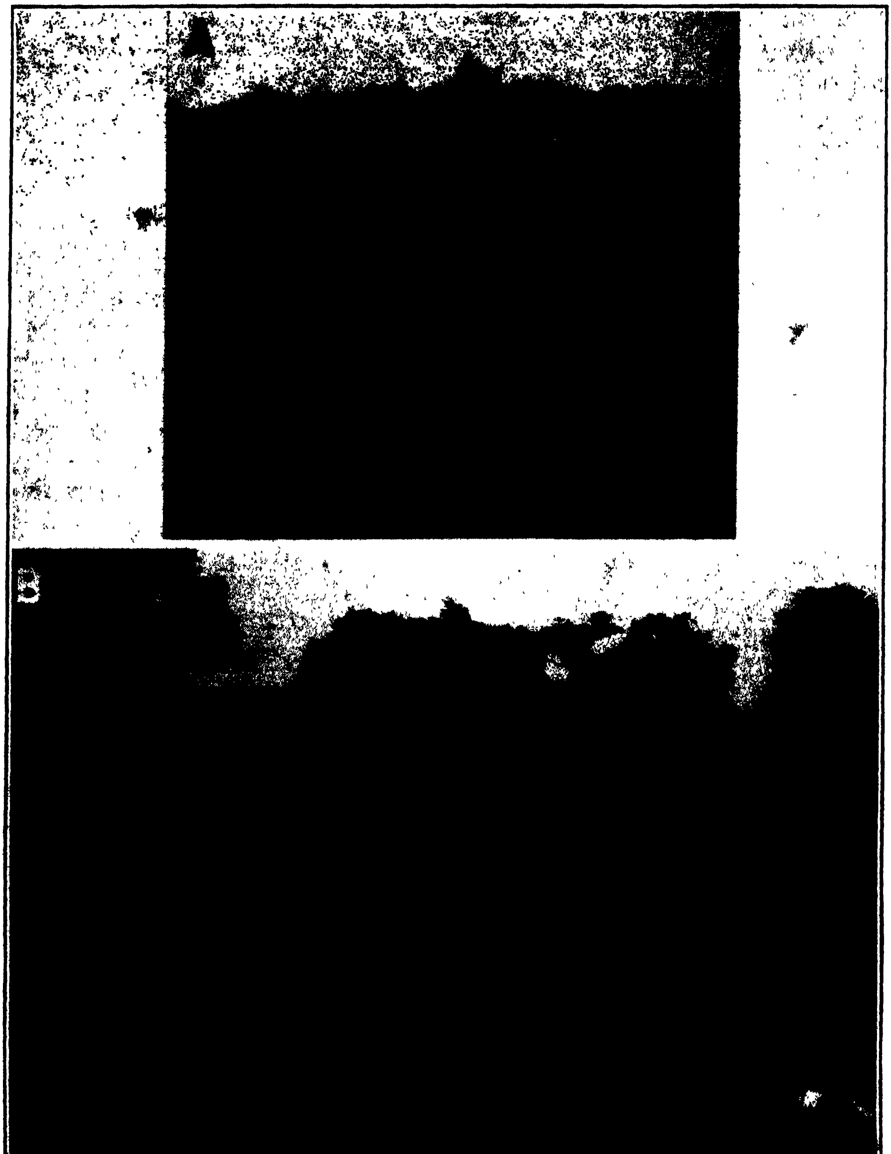

FIG. 2. A. A typical backyard, 15-foot row of badly blighted 3-year-old topset onions growing within ¼ mile of commercial fields in Wayne County. B. An exceptionally large planting of 6-year-old topsets badly diseased and within a mile of commercial onions in Wayne County.

of a $\frac{1}{10}$-acre plot of bulbs grown for seed. In one end of it mildew was far advanced. Conidia evidently had been produced here for several weeks (Fig. 1). Still farther up the hillside, a quarter of a mile northwest of these onions, there was a backyard planting of topsets in a similar advanced state of blight. This also had been shedding spores for several weeks (Fig. 2, A). Since the prevailing winds are from the northwest, they naturally

would carry inoculum in the direction of the commercial fields and distribute it in diminishing amounts as the distance increased. The counts of lesions and distances from the first-mentioned source are given in table 2.

TABLE 2. *Distribution of onion mildew in a valley 3300 feet long. July 8, 1937*

Field	Distance from primary source	Mildew lesions per 100 ft. of row
1	120 feet	1135
2	780 "	107
3	1750 "	4
4	2200 "	1.7
5	2800 "	1.0
6	3300 "	.33

To obtain an idea regarding the potential number of spores that a diseased backyard planting could produce, the following counts and computations were made. First, the number of spore-bearing tips of 63 conidiophores was counted under the microscope. The average number was found to be 16. The number of stomata per square inch of onion leaf surface was then calculated after a few counts and found to be approximately 65,000. If we then postulate 1 conidiophore growing out from but 1 of every 10 stomata, we have a total of over 100,000 conidia per square inch of leaf surface. It is, of course, common for the fungus to sporulate over several square inches of a plant at one time, so that a single small planting of a few dozen diseased onions can give rise to many millions of conidia overnight and can repeat this production many times in the spring. The writer has wiped a crop of conidiophores from a given leaf as many as 3 times, and each time a new crop formed within 48 hours.

THE EXTENT OF PERENNIAL ONION PLANTINGS IN ONE COUNTY

A survey was made of some 95 miles of county road across Wayne County, New York, the last week of June. A total of 497 farm properties were examined for backyard plantings of onions. Only 240 of these had vegetable gardens, but topsets were found in 17 of them, 8 of which were diseased. In most cases the diseased plantings were closer to the mucklands than were the healthy ones.[4]

Similarly, in the town of Marion, possessing approximately 175 home properties, most of which had backyard vegetable gardens, a survey in June showed that, out of 60 gardens examined, topset and multiplier onions were growing in 21 of them, and mildew was present in 17 of these 21. Here was a potential source of many millions of spores not over a mile or two from important commercial plantings. It is of interest to note that, in 1937, fields of onions to the west of Marion were much less injured than fields to the east. The prevailing winds are from the west, which fact may have a bearing on this.

[4] Almost all commercial onion culture in upstate New York is on muckland.

Besides the backyard plantings noted above, in Wayne County, over 50 others have been located on a few main roads in the western part of the State. Of these, 31 were diseased by July, 1937 (Fig. 2). If the ratio of perennial plantings per square mile holds for the entire State, there must be many hundreds if not thousands of these hidden sources of primary inoculum.

PROOF OF AERIAL DISSEMINATION OF CONIDIA

Two separate experiments were performed in midsummer to demonstrate that the conidia of this fungus were floating in the air and that they were viable. On July 15, a series of 37 stationary spore traps were mounted about 3 to 5 feet above ground on fence posts, in shrubs, and on lath supports in a pasture extending ⅓ mile north of a severely infected field of onions. They were left overnight, during which time a trace of precipitation occurred, and a favorable 12-mile wind blew from the south. The next morning the 37 slides were collected and subsequent examination revealed the presence of conidia of *Peronospora* on 12 of them. The conidia were easily detected by their great size. Four of them were caught the maximum distance of ⅓ mile from the nearest onions. There is no proof that any of these were viable when caught, but some indication was afforded by the fact that similar spores collected on leaves at 6 p.m., a few minutes after setting up the traps, were observed to germinate overnight. It is, therefore, likely that a certain proportion of those in the air were also viable. The day had been cloudy, misty, and altogether very favorable for sporulation.

To obtain further evidence on the aerial dissemination and viability of these conidia, on August 1 a flight was made by airplane over a heavily infected onion-growing area, known as the Montezuma Marshes, lying 50 miles north of Ithaca.[5] Plain agar Petri-dish spore traps, taken up in autoclaved sealed cans, were exposed for 4-minute periods at different elevations between 1500 and 50 feet (Fig. 3). The speed of the plane was held down to approximately 55 miles an hour, so that 9 square inches of agar surface in each trap had a chance to come in contact with approximately 1200 cu. ft. of air. Exposures were made between 9:25 and 10:05 a.m., with a temperature of 20° C., and relatively humidity of 85 per cent. A heavy fog, blanketing the ground all night, had induced abundant sporulation; and a light northwest wind rose to approximately 8 miles an hour by 10 a.m.

The traps were taken to the laboratory and incubated overnight at 15° C. before examination. The results of the flight and the subsequent spore germinations are given in table 3.

Conidia of the *Peronospora* in question were caught at all elevations from 1500 feet down. Although there was considerable thinning out above 700 feet, doubtless they could have been caught much above 1500 feet if sufficient search had been made in the right places. Probably the most significant

[5] The writer is indebted to Mr. H. M. Peters, manager of the Ithaca Flying Service, for his interest and aid in prosecuting these studies; to Mr. Jack Hoffman, the pilot; and to Mr. F. C. Meier of the U. S. Department of Agriculture for the loan of some of the "skyhook" equipment used by Col. Lindbergh in 1933.

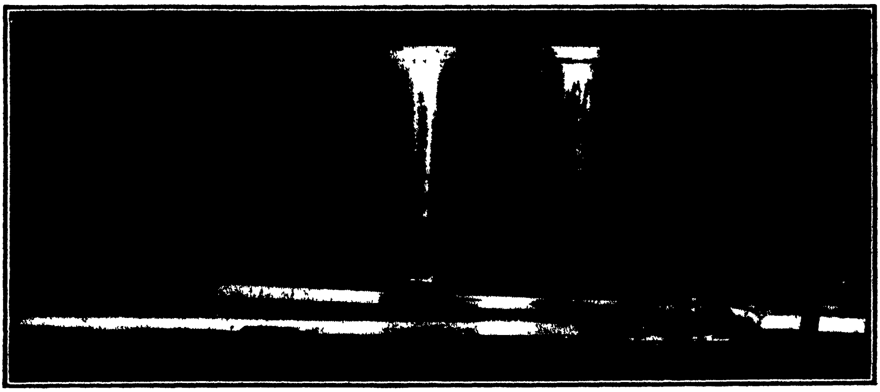

FIG. 3. Petri-dish spore traps and wooden holders (foreground) used in airplane flight over onion fields. Note parallel lines ruled on bottom of dish to facilitate microscopic examination later. The dishes in sealed cans were removed as needed through a slit near the bottom of the vertical cans in the background.

TABLE 3. *Results of airplane spore-trap exposures made August 1 to catch conidia of Peronospora destructor. Savannah, New York, Aug. 1, 1937*

Trap No.	Elevation in feet	Total conidia of *Peronospora* caught	Number germinating	No. cu. ft. of air per spore[a]
1	1500	7	6	171
2	1200	1	1	1200
3	1200	3	2	400
4	700	1	1	1200
5	700	13	11	93
6	700	11	5	109
7	200	30	30	40
8	100	26	15	46
9	50	37	26	32
10	[b]	3	3	400
11	2000[c]	0	0	0
	Total	132	100	

[a] Calculated by assuming each trap acted as a filter to extract the spores from 302 cu. ft. of air per minute. If the spores had been as large as hen's eggs, they would have blotted out the sun and have made flying through them too dangerous to attempt.

[b] Exposed on leaving the field and climbing to 1000 feet.

[c] Exposed over end of Cayuga Lake 5 to 10 miles from field.

fact was that 100 of the 132 conidia caught promptly germinated on the agar during the next few hours. This seems to be the first report of viable conidia of one of the Peronosporales being secured from even these low elevations.

In addition to the conidia of *Peronospora,* many other spores were in the air, as found by several others, who have captured spores in similar manner, viz., Stakman, *et al.* (11), Meier, Stevenson, and Charles (8), and Meier and Lindbergh (7). Spores of *Macrosporium* or *Alternaria* spores were the most abundant. Urediospores of rusts, pollen grains, and conidia of *Helminthosporium,* smuts, and various molds, such as *Penicillium* and *Aspergillus,* also were obtained.

An attempt was made to infect several potted onion plants by inoculation with germinated conidia transferred from the agar spore traps, but without success. Even the control plants inoculated with a fresh spore suspension failed to develop mildew, probably because of the exceptionally hot weather that prevailed during the two weeks immediately following inoculation.

LONGEVITY OF CONIDIA UNDER CONTROLLED TEMPERATURE AND HUMIDITY CONDITIONS

The question of the length of time *Peronospora* conidia can remain viable in the air under a variety of temperature and humidity conditions has an important bearing on the general question of aerial dissemination of the conidia and the need for eradication of perennial onions. Earlier investigators (2, 13) in this country have emphasized the short life of these spores. indicating it to be only a few hours at most, although Angell and Hill (1) state that in one test in Australia 2 per cent of the conidia remained viable on glass slides out of doors for 24 hours, and, in Russia, Katterfeld (4) states that he kept some alive for 10 days in a moist atmosphere.

The method of MacLachlan (6) was used employing Kolle culture chambers with sulphuric acid solutions in them to maintain the spores at 6 different relative humidities between 50 per cent and 100 per cent, inclusive. Spores were collected from topset onions in the writer's garden, in the morning, and brought to the laboratory on ice while still moist with dew. They were transferred to glass slides, then were placed in the Kolle flasks and held at different constant temperatures for varying periods of time, up to 5 days, when the slides were removed, drops of water placed on them and incubated for at least 12 hours at 12° C. The results of these spore germination tests showed the conidia capable of remaining viable longer and at lower humidities than were expected. It is regrettable that the experiments were stopped by cold weather, which cut off the supply of conidia before final results could be obtained, but a summary of the data, as far as they go, is presented in table 4, which shows that some spores were able to survive for several days at humidities above 70 per cent and ordinary temperatures, such as prevail in the spring for many days at a time (Fig. 4).

As it was never possible to obtain spores enough from any one lesion for an entire series of slides, it is not surprising that considerable variability in viability was encountered. Spores of *Peronospora tabacina* from different lesions and from leaves of different ages have also been found by Angell and Hill (1) to lack uniformity in vitality.

EFFECT OF SUNSHINE ON VIABILITY OF CONIDIA

Sunshine has been considered by some to be deadly to the conidia of *Peronospora,* but it seems that when the factors of temperature and humidity are favorable, the spores can withstand an exposure of several hours to bright sunshine. This was determined by exposing drops of spore suspensions on glass slides to the direct rays of the sun on the laboratory window-

FIG. 4. A. Germination of conidia of *P. destructor* caught on plain agar 1500 feet above an onion field August 1, 1937. The germ tube of the one on extreme left is not in focus. × 85. B. Conidia germinating 48 per cent after 60 hours exposure to air at 70 per cent relative humidity, in the dark, at 15° C × 85

sill. Preliminary tests indicated that exposures up to 2 hours were harmless. On September 25, which was an exceptionally clear, bright day, a 7-hour exposure was made. To insure a favorable temperature, the slides were placed on an aluminum tray of ice cubes The temperature of the

TABLE 4.—*Viability of Peronospora destructor conidia after exposure to various temperature and humidity conditions*[a]

Exposure period	Relative humidity 100 %	90 %	80 %	70 %	60 %	50 %	Exposure period	Relative humidity 100 %	90 %	80 %	70 %	60 %	50 %
9° C.							15° C.						
48 hrs.	36.0	78.0	68.0	11.0	tr.	0	4 hrs.	72.0	48.6	37.3	22.0	32.6	2.0
84 "	83.0	75.0	56.0	23.0	3.0	0	14 "	82.6	67.8	37.0	23.0	tr.	0.0
120 "	30.0	23.0	29.0	0.5	0.0	0	30 "	76.8	81.0	85.2	80.0	11.4	0.0
							60 "	71.0	90.6	77.4	47.8	1.1	
							72 "	81.6	41.2	38.8	2.2	tr.	tr.
							84 "	54.0	40.0	31.6	5.0	0.2	0.0
21° C.							27° C.						
6 hrs.	97.0	79.6	64.8	48.2	1.2	tr.	12 "	48.0	48.6	37.0	3.4	0.8	-
12 "	86.2		53.4		13.0		24 "	47.0	13.4	2.1	tr.	tr.	
24 "	6.8	82.8	12.6	5.8	0.3								
48 "	9.4	6.0	2.0	0.0	0.8								
60 "	18.0	5.0	tr.	4.2	0.0								

[a] Percentage germination figures in the body of the table are means of 5 counts of 100 spores each.

air in the bright sun ranged between 21° and 24.5° C., but the temperature of the slides remained between 13° and 20° C. Control slides were maintained just inside the window in the shade. The drops of water had to be renewed about every 30 to 60 minutes to take care of evaporation. Germination counts (Table 5) were made the next day after an incubation period overnight at 12° C.

These tests simply indicate that sunshine, in itself, is not lethal to conidia of *Peronospora* if they do not become dried or too warm. Katterfeld (4) reported that *P. destructor* conidia did not survive 2 hours' exposure to sun in a dry atmosphere, but Angell and Hill (1) obtained slight germination after 24 hours' exposure on a glass slide, part of which time was said to have been spent in direct sunlight.

TABLE 5.—*Germination of conidia of Peronospora destructor after exposure to sunshine in drops of water*

Exposure	Mean germination	
	In shade	In sunshine
	Per cent	*Per cent*
Checks—in incubator directly	76.0	
1 hour	85.4	78.0
2 hours		63.8
3 "	69.0	75.0
4 "		78.0
6 "		27.3
7 "	72.6	6.9

EFFECT OF LOW TEMPERATURES

Freezing the conidia of *Peronospora destructor* was found to lower the germination but not to destroy it entirely. After 12 or 15 hours in a block of ice, approximately 12 per cent germination was obtained in one trial. In this connection, Patel (10) reported germination of *P. trifoliorum* after 173 hours in a frozen condition, and Leach (5) found that not only were conidia of *P. schachtii* unaffected by short periods of freezing but that a few were capable of germinating after 40 days at –12° C.

VIABILITY IN DILUTE COPPER SOLUTIONS

It has always been thought that copper sulphate was extremely toxic to the spores of the Peronosporales, but McWhorter and Pryor[6] have pointed out that malachite green is much more so. The writer, too, found it almost twice as toxic as copper sulphate. The dye (light green N) completely inhibited germination at dilutions up to 1–150,000, while copper sulphate permitted germination at dilutions greater than 1–75,000.

Whether control by spraying will ever be successful, even with better fungicides, is still a question and beyond the scope of the present work. Several years ago great efforts were made to hold the disease in check in New

[6] McWhorter, F. P., and J. Pryor. Onion mildew in Oregon and the advisability of testing malachite green as a control agent for downy mildews. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 21: 306–307. 1937. [Mimeographed.]

York with Bordeaux mixture and copper-lime dust applied thoroughly and frequently, but with no success whatsoever.

DISCUSSION

Without reference to the possible transmission of onion mildew through the seed, which may or may not take place, it seems to the writer that further progress on that phase of the problem must now await the solution of a more pressing problem of eradicating the disease early in the spring by destruction of the many perennial sources of primary inoculum that exist as diseased backyard plantings of perennial topset and multiplier onions. There are far more of these plantings in the East than have been suspected. Some plantings have been in for over 40 years. They are on the increase, and there is reason to believe that a very large proportion (over one-half) of them in the commercial onion-growing counties are diseased.

It has been shown that the conidia of *Peronospora destructor* can be recovered from the air several hundred feet off the ground in a viable condition and that the spores are well able to remain viable for several days at temperatures and humidities that often prevail in the spring for many days in the Northeastern States. Thus, all the necessary conditions for successful aerial dissemination seem to be met.

In this connection, Doran (3) pointed out that the spread of downy mildew of cucurbits into the New England States is most logically explained on the assumption that winds and rain bring the conidia of *Peronoplasmopara cubensis* up the coastal States from Florida. Similarly, Leach (5) presents evidence to indicate that the spread of downy mildew of beet in California takes place over restricted areas by means of wind-borne conidia. The writer has been informed that there is good circumstantial evidence that *Peronospora tabacina* on tobacco is carried up with winds accompanying rainy periods from certain sources of primary infection in Florida into Georgia and the Carolinas. If the fungi causing these are wind-borne, then those causing late blight of potato and several other downy mildews may also be, to a considerable extent.

How far from commercial plantings it will be necessary to eradicate perennial onions to safeguard the commercial crop is still another problem; but, from the work on rusts of apple and white pine, one might think 3 to 5 miles would be sufficient in most seasons. As others have pointed out, however, there is no secondary spread in the case of the two rusts mentioned, while such infections are very common and important in the case of the mildews. On theoretical grounds, a field 2 miles distant from a given source of primary inoculum should be 4 times as safe as a field 1 mile distant, but this is true only when spores are evenly distributed in all directions.

The problem of overwintering in regular onion sets and in bulbs to be used for growing seed is still a very important one. But it can probably best be solved through bulb treatment after the manner described by Murphy and M'Kay (9). Work along this line is in progress; but, even if successful, the results may be more or less nullified unless the backyard plantings of diseased perennial onions are eradicated, at least in the im-

portant commercial onion districts of the North. We have been able to confirm Murphy and M'Kay's discovery of the mycelium of *Peronospora destructor* in the bulbs of topset onions and expect to report on bulb-treatment experiments in the near future.

SUMMARY

Conidia of *Peronospora destructor* were caught in the air over diseased onion fields to a height of 1500 feet. Of 132 trapped on one flight of 40 minutes, 100, or 75 per cent, germinated.

Conidia have been found to survive freezing, exposure of 7 hours to bright sunshine in drops of water, and many days' exposure (at least 5) to air in the dark at a temperature of 9° C. and a relative humidity above 70 per cent.

Perennial topset and multiplier onions have been found commonly growing in backyard gardens on farms and in the small towns of agricultural counties. A large proportion of these were found to be diseased in June. Mycelium has been found in bulbs in November.

The conclusion is drawn that in some seasons, at least, these diseased perennial onions are a distinct menace to the $2,000,000 commercial crop of onions grown in New York State, because mildew is evidently a wind-borne disease.

Malachite green was found much more toxic than copper sulphate to the conidia.

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A MOSAIC-RESISTANT SMALL RED BEAN[1]

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(Accepted for publication January 20, 1938)

INTRODUCTION

The common bean mosaic disease is very destructive to the small red or Red Mexican variety of beans in southern Idaho. Because of its resistance to curly top, the Red Mexican variety of beans constitutes an important part of the bean crop grown in Twin Falls and adjacent counties in local areas where, the beet leaf hopper, *Eutettix tenellus* Baker, is prevalent. According to a mimeographed report of the United States Department of Agriculture,[3] Idaho produced 250,000, 100-pound bags of small red beans in 1935 and 164,000 in 1936. The total small red-bean production in the United States was 292,000, 100-pound bags in 1935 and 190,000 in 1936. Common bean mosaic has been recognized for many years and has been the subject of study by several workers. Pierce[4] described several properties of the common bean mosaic virus (*bean virus 1*) and reported yellow bean mosaic virus (*bean virus 2*). Common bean mosaic is a seed-borne disease that may be transmitted in the field to susceptible plants by several species of aphids. Important pathological effects of this disease on the Red Mexican variety include mottling and curling of leaves, dwarfing of the plant with a reduction in the yield and delayed maturity of the crop. The Red Mexican bean is very susceptible to this disease, and Idaho growers experience severe losses every year.

Both public and private agencies have been interested in the development of bean varieties tolerant or resistant to, the common bean mosaic disease. The Robust variety was introduced by the Michigan station in 1913 and was later shown by Reddick and Stewart[5] to be resistant to common bean mosaic. In 1934 Pierce and Walker[6] introduced 2 mosaic-resistant Refugee beans—Idaho Refugee and Wisconsin Refugee. The United States Department of Agriculture has developed U. S. No. 1, a garden variety tolerant to common bean mosaic, and U. S. No. 5, a Refugee type garden bean, resistant to common bean mosaic. The Plant Pathology Department of the University of Idaho has for several years conducted a breeding program for mosaic resistance in various varieties of beans and has developed and introduced 4 strains of Great Northern beans resistant to the common mosaic disease.

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2 W. H. Pierce was formerly Associate Plant Pathologist of the Idaho Agricultural Experiment Station. The writers wish to express their deep appreciation to Dr. C. W. Hungerford for his helpful suggestions throughout the course of this investigation and for aid in the preparation of the manuscript.

3 United States Department of Agriculture, Bureau of Agricultural Economics. Production of beans in the United States, by commercial classes 1919–1936. 10 pp. Jan., 1937. [Mimeographed.]

4 Pierce, W. H. Viroses of the bean. Phytopath. 24: 87–115. 1934.

5 Reddick, D., and V. B. Stewart. Varieties of beans susceptible to mosaic. Phytopath. 8: 530–534. 1918.

6 Pierce, W. H., and J. C. Walker. The development of mosaic-resistant Refugee beans. Canner 77(26): 7–9. 1933.

The first to be developed was Great Northern U.I. 1, which was followed by U.I. 81, U.I. 59, and U.I. 123. The three strains, U.I. 81, U.I. 59, and U.I. 123, have replaced common Great Northern and U.I. 1 in commercial production, and now constitute almost the entire crop of Great Northern beans grown in Idaho. This paper deals with the development and introduction to the Idaho growers of a common-bean-mosaic-resistant Red Mexican variety.

MATERIALS AND METHODS

Throughout the program for the development of bean varieties resistant to common bean mosaic, extensive field trials were conducted in southern Idaho. The field trial plots were located near Buhl, where curly top was severe, and near Twin Falls, where the mosaic disease was severe. Several field selections were made in the Great Northern variety and from these selections strains of mosaic-resistant Great Northern beans were developed. Field selections in the Red Mexican variety failed to produce a resistant type; therefore, the mosaic-susceptible Red Mexican was crossed with a mosaic-resistant parent.

In 1929 several crosses and reciprocal crosses of Red Mexican and mosaic-resistant Great Northern U.I. 1 were made. The purpose of this particular cross was twofold: first, to aid in the development of a Red Mexican variety resistant to common bean mosaic; second, to aid in the development of a Great Northern variety resistant to curly top.

Since 1929 a continuous program of plant selection and testing in field trial plots was conducted with the material from the Red Mexican-Great Northern cross. Data were obtained on plant types, curly-top and mosaic-disease resistance of a large number of selections. Two selections, No. 3 and No. 34, proved most valuable from several standpoints. These two selections were increased until a substantial amount of seed was ready for distribution to the bean growers of southern Idaho.

YIELD AND QUALITY OF RESISTANT SELECTIONS

Throughout the program for the development of a Red Mexican variety resistant to common bean mosaic, an effort was made to obtain those selections that were of value because of their yield and their vine and seed characteristics. Two selections, No. 3 and No. 34, are very similar in seed size to the Red Mexican parent, and this small size of seed seems to be a characteristic demanded by the trade. The seed-coat color of selections No. 3 and No. 34 is darker red than the Red Mexican, a deviation in color thought to be an improvement of the Red Mexican variety. Soaking and cooking tests were made with the new selections and the Red Mexican parent, and it was found that the new selections retained more red color after soaking than did the parent types. Cooking tests showed that the new selections were of equal quality to the Red Mexican variety.

Vine and leaf characters of the new selections are very similar to each other and, in general, very similar to the Red Mexican parent, although Red

Mexican plants have foliage of a darker shade of green than that of the new selections. In comparing the two selections it was observed that selection No. 3 is of a slightly heavier vine structure and is somewhat darker green in color than No. 34. On limited field-plot tests the mosaic-resistant selections have consistently produced a higher yield than the Red Mexican parent. In table 1 are presented yield data obtained in 1936 and 1937, together with disease data of the two selections and the Red Mexican variety. In the new mosaic-resistant Red Mexican variety the inherent curly-top resistance of the Red Mexican variety has been retained with the mosaic resistance obtained from Great Northern U.I. 1.

TABLE 1.—*Data on yield and disease counts of two mosaic-resistant hybrid selections and Red Mexican beans*

Selection	Stand 50′ row		Common mosaic		Curly top		Yield of 50′ of 4 rows		Average yield
	1936	1937	1936	1937	1936	1937	1936	1937	
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Lbs.*	*Lbs.*	*Lbs.*
U.I. 3	129	131	0	0	0	0	29.5	35.7	32.6
U.I. 34	103	164	0	0	0	0	31.3	35.3	33.3
Red Mexican	202	168	70[a]	90[a]	0	0	18.8	29.0	23.9

[a] Estimated infection.

It should be stated that neither Red Mexican, Great Northern U.I. 1, nor the two new hybrid selections are resistant to yellow bean mosaic (*bean virus 2*). Yellow bean mosaic and common bean mosaic can easily be confused in the initial stages of development under field conditions. Yellow bean mosaic is not seed-borne and is of only minor importance in the commercial production of small red beans in Idaho.

PLANS FOR RELEASE

It is sometimes a difficult problem in commercial bean production to keep a variety pure. Growers will no doubt find this to be true, as the new strains were distributed in a bean-growing section where large plantings of small red beans have been produced for several years. While the new strains were being grown in the increase plots, they were inspected by the State Seed Inspector in the regular manner. Later, after passing further tests, they were certified and sealed. The new strains were distributed to selected growers through the cooperation of the Idaho county extension agents. Growers will be encouraged to meet the high standard of the State inspection service with the hope that a large seed supply can be obtained free from varietal mixture.

NAMES OF THE RESISTANT SELECTIONS

The two new common-bean-mosaic-resistant selections have been named Red Mexican U.I. 3 and Red Mexican U.I. 34. The numbers, in each case,

represent the nursery number of each selection. Red Mexican will designate the variety and distinguish the new small red beans from other varieties developed by the University of Idaho. This method of naming follows the one used with the Great Northern varieties resistant to common bean mosaic.

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FURTHER DETERMINATIONS OF THE CARBOHYDRATE-NITROGEN RELATIONSHIP AND CAROTENE IN LEAF-HOPPER-YELLOWED AND GREEN ALFALFA[1]

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(Accepted for publication February 5, 1938)

INTRODUCTION

In an earlier paper[3] the writer reported that alfalfa leaves yellowed by the potato leaf hopper, *Empoasca fabae* (Harris), are lower in nitrogen and higher in carbohydrates than green alfalfa leaves. The samples for the analytical work upon which this conclusion was based were produced in infested and noninfested cages of cheesecloth or tobacco cloth. More recently[4] he has reported that alfalfa yellowed by natural infestation with leaf hoppers is much lower in carotene, and, hence, in potential vitamin A activity, than green alfalfa kept free from leaf hoppers by dusting with a mixture of 300-mesh sulphur and pyrethrum. A third possible method of producing samples of leaf-hopper-yellowed and green alfalfa for comparative analysis would be to cage a portion of a plot after the first cutting was made and allow the remainder of the plot to be subject to natural infestation with leaf hoppers. In this setup, the cage used to protect a portion of the alfalfa from infestation might be considered as taking the place of the protective dust used in method 2. It seemed desirable to determine if these different methods of producing leaf-hopper-yellowed and green alfalfa would yield samples of comparable chemical composition insofar as the carbohydrate-nitrogen relationship and carotene content were concerned.

[1] This paper reports the results of one phase of a cooperative study of the injury by *Empoasca fabae* (Harris) to forage legumes being made at Arlington Experiment Farm, Arlington, Va., by the Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Indebtedness is acknowledged to J. G. Conti, formerly Junior Biochemist, Bureau of Plant Industry, for assistance with the carbohydrate and nitrogen determinations and to C. A. Cary and H. G. Wiseman, Bureau of Dairy Industry, U. S. Department of Agriculture, for making the carotene determinations on samples submitted by the writer.

[3] Johnson, H. W. Nature of injury to forage legumes by the potato leaf hopper. Jour. Agr. Res. [U. S.] 49: 379–406. 1934.

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EXPERIMENTAL METHODS AND RESULTS

In July, 1932, samples of leaf-hopper-yellowed and green Peruvian alfalfa leaves were harvested from noncaged plants and from plants in a noninfested cheesecloth cage and were preserved in alcohol for analysis. In August, 1932, similar samples were harvested from an infested and a noninfested cage in the same plot of Peruvian alfalfa as above. In July, 1935, samples were harvested from nondusted Grimm and Kansas alfalfa

FIG. 1. Shoots of leaf-hopper-yellowed Grimm alfalfa (left) from a nondusted plot compared with shoots of green Grimm alfalfa (right) from a plot kept relatively free from leaf hoppers by dusting with a mixture of 300-mesh sulphur and pyrethrum. Samples for chemical analysis were produced in this way for comparison with samples produced in infested and noninfested cheesecloth cages.

plots in which the alfalfa was heavily infested with leaf-hoppers, stunted, and badly yellowed, as shown in figure 1, and from adjacent dusted plots of the same varieties in which the alfalfa was green and relatively free from leaf hoppers. All of these samples subsequently were analyzed for carbohydrates and nitrogen by the methods reported in the earlier paper.[5] The results of these analyses are presented in table 1. These data show that the leaf-hopper-yellowed alfalfa leaves, whether from infested cages or cage-free and nondusted plots subject to natural infestation, are higher in dry matter,

[5] See footnote 3.

TABLE 1. *The carbohydrate-nitrogen relationship in leaves of alfalfa plants yellowed by natural infestation with leaf hoppers and by artificial infestation in a cheesecloth cage compared with the relationship in leaves of green alfalfa plants kept free from leaf hoppers by cheesecloth cages and by dusting with a mixture of 300-mesh sulphur and pyrethrum, Arlington Experiment Farm, Arlington, Virginia, 1932–1935. Results are presented for duplicate samples in all cases, except the noncaged rows in 1932. All carbohydrates are reported as dextrose and the percentages given for these are averages of duplicate determinations on each sample*

Plant material	Source	Dry matter	Green weight: Reducing sugar	Green weight: Total sugar	Green weight: Total acid-hydrolyzable	Green weight: Total nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Peruvian alfalfa leaves July, 1932	Noninfested cage	27.27	0.19	0.76	4.07	1.00
		24.37	0.18	0.62	2.46	0.97
	Noncaged rows	37.81	0.90	1.95	8.71	0.90
Peruvian alfalfa leaves Aug., 1932	Noninfested cage	25.92	0.19	0.66	3.11	1.18
		26.16	0.22	0.72	3.29	1.20
	Infested cage	37.20	1.20	2.65	10.60	1.05
		39.70	1.35	2.64	11.01	1.14
Grimm alfalfa leaves July, 1935	Dusted plot	29.00	0.24	0.80	3.29	1.12
		28.90	0.28	0.79	2.92	1.10
	Nondusted plot	36.73	1.05	1.87	9.31	1.03
		37.71	1.11	1.87	9.17	1.04
Kansas alfalfa leaves July, 1935	Dusted plot	28.40	0.23	0.71	3.77	1.10
		28.60	0.25	0.73	4.12	1.10
	Nondusted plot	36.75	0.98	1.82	9.62	1.02
		37.97	0.99	1.80	9.64	1.02

reducing sugar, total sugar, and total acid-hydrolyzable substances than are green alfalfa leaves from noninfested cages or dusted plots. On the other hand, the yellowed alfalfa leaves are lower in total nitrogen in all cases than are the green ones. The constancy of these differences in all the pairs of samples and their agreement with previous analyses of yellowed and green alfalfa leaves,[6] indicate that either caging certain plants or protecting them by means of sulphur-pyrethrum dust is a satisfactory method of producing samples free from leaf-hopper injury for a determination of the chemical differences between noninjured material and alfalfa subject to natural infestation with leaf hoppers or produced in artificially infested cages.

Samples of the stems of plants from the nondusted and dusted plots of Grimm and Kansas Common alfalfa also were preserved in alcohol and analyzed for carbohydrates and nitrogen at the same time as were the leaf samples. The results of these analyses are presented in table 2. These data show that the stems of leaf-hopper-yellowed alfalfa plants (nondusted) are lower in dry matter, reducing sugar, total sugar and total acid-hydrolyzable substances than are the stems of green alfalfa plants from dusted plots. On

[6] See footnote 3.

TABLE 2. *The carbohydrate-nitrogen relationship in stems of alfalfa plants yellowed by natural infestation with leaf hoppers compared with the relationship in stems of green alfalfa plants kept free from leaf hoppers by dusting with a mixture of 300-mesh sulphur and pyrethrum, Arlington Experiment Farm, Arlington, Virginia, 1935. Results are presented for duplicate samples in all cases. All carbohydrates are reported as dextrose and the percentages given for these are averages of duplicate determinations on each sample*

Plant material	Source	Dry matter	Green weight			
			Reducing sugar	Total sugar	Total acid-hydrolyzable	Total nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Grimm alfalfa stems July, 1935	Dusted plot	34.06	0.31	0.98	6.75	0.66
		34.33	0.28	0.96	7.02	0.67
	Nondusted plot	33.05	0.17	0.62	6.73	0.76
		33.42	0.16	0.55	6.53	0.74
Kansas alfalfa stems July, 1935	Dusted plot	34.85	0.26	0.97	6.88	0.61
		35.08	0.26	1.04	7.18	0.60
	Nondusted plot	34.06	0.22	0.73	6.59	0.80
		34.27	0.19	0.67	7.02	0.75

the other hand, the yellowed stems are higher in total nitrogen than the green alfalfa stems.

It is evident from a comparison of the data presented in tables 1 and 2 that the carbohydrate-nitrogen relationship in yellowed and green alfalfa leaves is just the reverse of that existing in yellowed and green alfalfa stems. The yellowed leaves are higher in carbohydrates and lower in nitrogen than are the green leaves, whereas the yellowed stems are lower in carbohydrates and higher in nitrogen than the green stems. The work of Granovsky[7] and of Smith and Poos[8] showed that the potato leaf hopper feeds upon the vascular tissues of its leguminous hosts and causes a clogging of the food-conducting elements. One would expect such injury to retard the translocation of elaborated food from the leaves back into the stems and to result in a congestion of carbohydrates in the leaves and a deficiency of these substances in the stems. The analyses presented show that this condition exists in the case of leaf-hopper-yellowed alfalfa.

Samples of noncaged second-cutting Grimm alfalfa that had yellowed badly and of green alfalfa from a caged area in the same small plot were preserved in a freezing mixture of absolute alcohol and solid carbon dioxide on July 15, 1937, and were taken the following day to the Nutrition Laboratory, Bureau of Dairy Industry, for determinations of the carotene content. The determinations, which were made spectrophotometrically, show that the green alfalfa contains 227 milligrams of carotene per kilogram of dry matter, whereas the yellowed alfalfa contains only 71 milligrams. The dry

[7] Granovsky, A. A. Differentiation of symptoms and effect of leaf hopper feeding on histology of alfalfa leaves. (Abstract) Phytopath. 20: 121. 1930.

[8] Smith, F. F., and F. W. Poos. The feeding habits of some leaf hoppers of the genus *Empoasca*. Jour. Agr. Res. [U. S.] 43: 267–285. 1931.

matter content of the two types of material was determined to be 26.62 per cent for the green alfalfa and 35.58 per cent for the noncaged, yellow alfalfa. These results confirm the analyses presented in a previous paper,[9] which were based upon samples of yellowed alfalfa from nondusted plots and of green alfalfa from dusted plots. It would appear, therefore, that in the case of carotene determinations either caging certain plants or protecting them by means of sulphur-pyrethrum dust is a satisfactory method of producing samples free from leaf-hopper injury for comparison with samples of alfalfa exposed to natural infestation with leaf hoppers.

SUMMARY

Leaf-hopper-yellowed alfalfa leaves, whether from infested cages or cage-free, nondusted plots, are higher in dry matter, reducing sugar, total sugar, and total acid-hydrolyzable substances but are lower in total nitrogen than are green alfalfa leaves from noninfested cages or dusted plots.

Stems of leaf-hopper-yellowed alfalfa plants from nondusted plots are lower in dry matter, reducing sugar, total sugar, and total acid-hydrolyzable substances but are higher in total nitrogen than are stems of green plants from dusted plots.

Leaf-hopper-yellowed second-cutting Grimm alfalfa (leaves and stems) from a noncaged area contains only 71 mg. of carotene per kg. of dry matter, whereas green alfalfa from a caged area, in the same small plot, contains 227 mg. per kg.

The constancy of results from these pairs of samples and their agreement with previously published data show that either caging certain alfalfa plants or protecting them by means of sulphur-pyrethrum dust is a satisfactory method of producing samples free from leaf-hopper injury for comparison with samples of alfalfa exposed to natural infestation with leaf hoppers or produced in artificially infested cages.

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[9] See footnote 4.

ASTEROID SPOT, A NEW VIROSIS OF THE PEACH[1]

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(Accepted for publication Nov. 20, 1937)

The extreme variation in the leaf expression of symptoms of peach mosaic in peach has given rise to difficulties in the identification of the disease, especially on partially tolerant varieties and under varied environmental conditions. In a study of such widely varying cases, a number of offtype leaf patterns have been found. · Some of them are sufficiently different to suggest mutation of the peach-mosaic virus, or the existence of distinct viruses capable of infecting the peach. Transmissible disorders, distinct from peach mosaic, but of the mosaic type, have been reported by Valleau[2] in Kentucky and Cation[3] in Michigan. Atanasoff[4] has described a mosaic on snow-white peach in Bulgaria, similar in some respects to the asteroid spot, but different from peach mosaic as described by Hutchins *et al.*[5] The purpose of this paper is to report and describe a transmissible disease affecting peach, which the authors believe is distinct.

HISTORY

The disease was first observed in 1934 on leaves of a root shoot of a hybrid peach tree, which had been developed in earlier peach breeding work at the Citrus Experiment Station. The seedling understock of this tree was grown and budded by a local nursery and the budded tree was planted in the station plot in 1919. In the process of the breeding experiment most of the trees were discarded, but some were saved and used as stocks for a prunus species nursery. One of these was grafted with scions of *Prunus alleghaniensis* in 1924. No abnormalities were noted in the grafted tree until 1934, except in one season when the leaves of the *P. alleghaniensis* developed numerous small dark purple spots, which were not associated with the present disorder. Upon the discovery of the condition, buds from the mottled shoots were placed in a seedling from an open pollinated S. P. I. *Amygdalus* Sp. No. 40001. The leaves on the peach shoots from the inserted buds developed typical spotting. The S. P. I. understock showed no spotting until 1937, when faint but easily recognizable spots developed on leaves near the graft union. This was indicative of the transmissible nature of the disease and attempts of further transmission were begun.

[1] Paper No. 384, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Valleau, W. D. A virus disease of plum and peach. Kentucky Agr. Expt. Sta. Bull. 327: 89–103. 1932.

[3] An unpublished paper entitled, "A rosetted-mosaic of peach and plum trees," by Donald Cation, was presented at the 1935–1936 A.A.A.S. meetings in St. Louis. The senior author was shown peach trees affected by the rosetted-mosaic virus by Mr. Cation in 1935 and the symptoms appeared different from the asteroid spot described in this paper.

[4] Atanasoff, D. Mosaic disease of drupaceous fruit trees. God. Sofiisk. Univ., Agron.-Lesov. Fakult. (Ann. Univ. Sofia, Facult. Agron. et Sylvic.) (V, 1934–35. 1, Agron.) 13: 9–42. 1935.

[5] Hutchins, L. M., E. W. Bodine, and H. H. Thornberry. Peach mosaic, its identification and control. U. S. Dept. Agr. Circ. 427. 1937.

SYMPTOMS AND NAME

The name asteroid spot has been assigned to the new malady because of the similarity of the leaf-spots to small star-shaped splotches. These spots appear as if small droplets of a thick yellow liquid had been thrown uni-

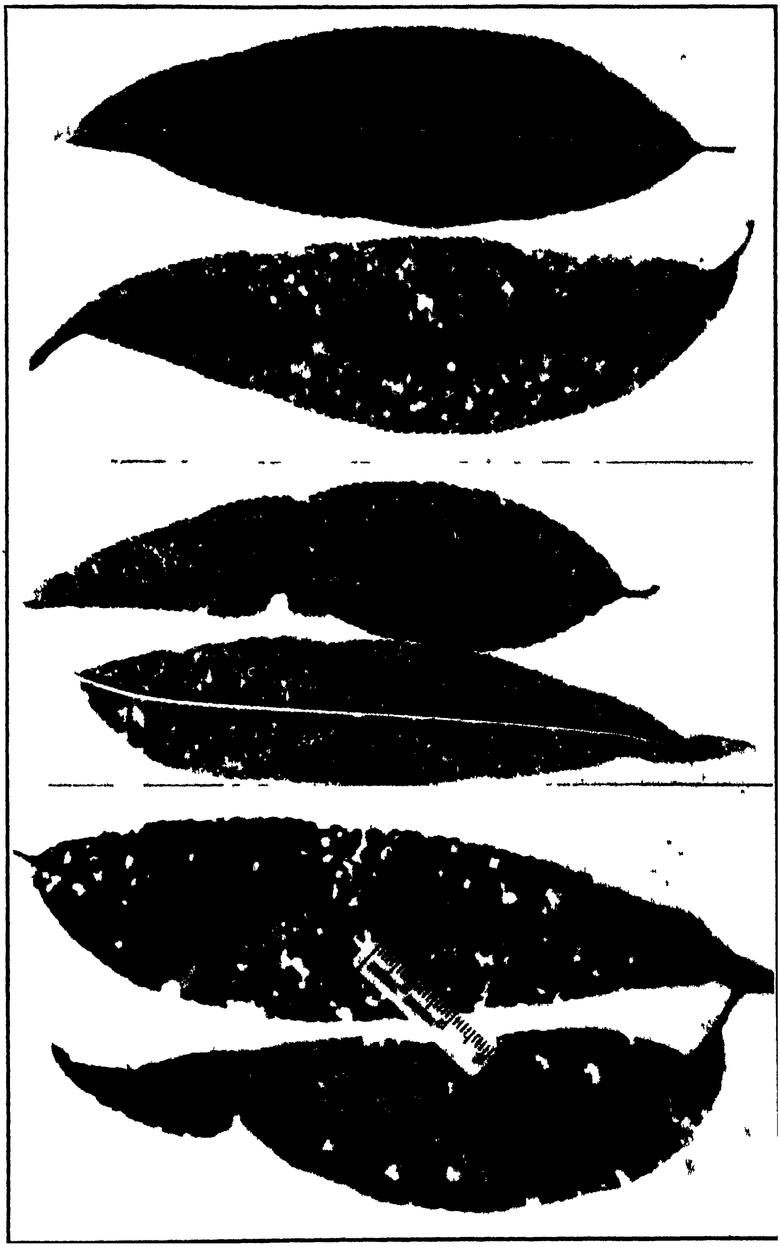

FIG. 1. Peach leaves showing typical leaf symptoms of asteroid spot. The four leaves at the bottom are from a naturally affected seedling peach. The two leaves at the top are from diseased and healthy J. H. Hale peach nursery trees. The diseased Hale leaf came from an artificially inoculated tree.

formly over the surface of the leaves and each droplet on incidence of hit had splattered in all directions (Fig. 1). The spots occur singly and vary in size with the number on the leaf. Newly formed leaves on the affected plants appear entirely normal, and the spots do not become evident until the leaves are fully expanded. On the darker green leaves the spots appear yellow on a green background, but as the leaves become older they turn prematurely yellow and reverse the color combination. The original fully formed spots are, in reality, light green, and do not change further, even though the leaf becomes entirely yellow. In the latter condition the spots appear light green on a yellow leaf. With the first development of yellow in the tissue surrounding the spots, affected leaves form abscission layers and are shed.

Three J. H. Hale peach-nursery trees, growing in 5-gallon cans in the greenhouse were grafted in February, 1937, with scions from peach affected with asteroid spot. After 8 weeks, fully expanded leaves on the Hale branches below the grafts had developed discernible spots. The disease progressed rapidly in one of the trees; and the tree shed its leaves and died. After 5 months the disease had invaded several branches below the grafts in the other inoculated trees, and older leaves had abscissed. Typical spots developed in leaves on shoots growing from the peach seedling understock of one of the inculated Hale trees, but were less intense than those on Hale peach leaves. The scions on all the trees grew for a short time, but later shed their leaves and died.

FIG. 2. Typical early summer leaf patterns of peach mosaic on J. H. Hale peach leaves.

Since the disease has not been seen on other than young shoots and nursery trees, the complete effects on peach are not known.[6] However, the trouble is recognized as a possible serious disease of peach, entirely unlike peach mosaic (Fig. 2), and should be handled only under controlled conditions. The writers plan to test the virus on other hosts, and then it will be destroyed.

CITRUS EXPERIMENT STATION,
UNIVERSITY OF CALIFORNIA,
RIVERSIDE, CALIFORNIA.

A BACTERIAL BUD AND STEM ROT OF ROCKET LARKSPUR[1]

P. A. ARK, C. M. TOMPKINS, AND R. E. SMITH

(Accepted for publication January 12, 1938)

A bacterial disease of rocket larkspur, *Delphinium ajacis* L., was observed in commercial seed fields in the spring of 1933 at Salinas, California. The disease occurred in localized areas but in subsequent years was more general in distribution, frequently killing a high percentage of the plants. Heavy infections always caused a marked reduction in yield of seed because of fewer plants at harvest time.

In 1935, Dodge[2] described a bacterial disease of *Delphinium ajacis* that caused a blackening and rotting of the aerial parts and stunting of the plants. Later, Chester[3] published an account of the causal organism, which he identified as *Erwinia phytophthora* (Appel) Comm. S.A.B.

SYMPTOMS OF THE DISEASE

In California, the disease was observed only on larkspur plants in the flowering stage. Characteristic symptoms consisted of a large number of yellow leaves, blackening of the stem, and stunting of the plants. The disease may start from the terminal buds or from the base of the lower leaves and spread downward through the stem. Invaded tissues are water-soaked and translucent at first, but later turn black. The bacteria are found principally in the cortical tissues, but, in later stages, they penetrate the vascular system. Diseased plants are not always killed but may survive infection and produce flowers and seed. Under moist conditions, however, the progress of the disease is rapid and invariably soft rot of tender plant parts ensues.

There is some evidence that the disease is carried in or on the seed. For

[6] Since this paper went to press, peach mosaic inspectors called the attention of the writers to a leaf spot on peach found in a commercial orchard. The planting was composed of several varieties, but the trouble was found only on the Red Bird variety. Although the spots were strikingly similar to those of asteroid spot, no premature defoliation was noted and damage on the affected trees was slight.

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California. Nontechnical assistance from employees under the Federal Works Progress Administration is acknowledged.

[2] Dodge, B. O. A bacterial disease of *Delphinium ajacis*. Jour. New York Bot. Gard. 36: 257–260. 1935.

[3] Chester, F. D. A bacterial disease of Delphinium. Phytopath. 27: 855–858. 1937.

example, seeds that were harvested in a field where the disease was prevalent were planted in autoclaved soil and occasionally gave diseased plants. Also, seeds collected from diseased plants in the field were treated in a 1-to-1000 solution of mercuric bichloride and planted in autoclaved soil in the greenhouse. Sometimes, diseased plants were observed.

The problem of insect transmission of the pathogen was investigated. Aphids, *Macrosiphum solanifolii* Ashmead, were caged on artificially infected larkspur plants for 5 days. Transfers were then made by means of a camel-hair brush in lots of 10 infective aphids to each of 25 healthy larkspur seedlings under cloth cages. After the aphids had fed on these plants for 2 days, the cages were removed and the plants fumigated. After four days, 5 plants became infected, showing typical symptoms of the disease. The organism was recovered in pure culture from each of these plants and proved to be identical with the stock culture. These limited tests indicate that, under certain favorable conditions, aphids may possibly be a factor in the natural spread of the disease.

THE CAUSAL ORGANISM

The causal organism described by Chester[4] was identified by him as *Erwinia phytophthora*. The taxonomic position of the organism responsible for the bacterial bud and stem rot of larkspur in California corresponds rather closely with *E. phytophthora*. However, it should be noted that there were some cultural differences between various isolates. Thus, some isolates did not reduce nitrates or utilize lactose. Some utilized glycerine in synthetic media with the production of acid and gas, while other isolates produced only gas. Hydrogen sulphide tests were positive for all isolates. These differences in the soft-rot group of organisms, observed by many investigators, were fully discussed by Leach.[5]

HOST RANGE

Young cultures of numerous varieties of *Delphinium*[6] were tested for susceptibility to infection by spraying them with a bacterial suspension or by puncturing the stems of large plants with a needle charged with bacteria. The following species and varieties of *Delphinium* were successfully infected: *Delphinium ajacis* L., *D. consolida* L., *D. elatum* L., *D. elatum* L. var. *duhinbergi*, *D. exaltatum* Ait., *D. geyeri*, *D. orientale* T. day Borisove, *D. perigrinum* L., *D. requienii*, *D. taitsiense*, *D. triste*, *D. hybridum* Prinz Gustav. *D. triste* was only slightly susceptible. The following varieties were grown from the seed and found to be susceptible: Giant Imperial Blue Bell, Blue Spire, Miss California, Daintiness, Carmine King, Lilac Spire, Los Angeles, New Lilac, White Spire, Stock-flowered Ageratum, Blue, Dark Blue, La France, Lustrous Carmine, Rosamund, Rose Queen, and White.

[4] See footnote 3.

[5] Leach, J. G. Blackleg disease of potatoes in Minnesota. Minnesota Agr. Expt. Sta. Tech. Bull. 76. 1931.

[6] The writers are indebted to Mr. C. O. Blodgett, Division of Genetics, who kindly supplied seeds of many varieties of *Delphinium* for these tests.

Carrot, *Daucus carota* L., roots and potato, *Solanum tuberosum* L., tubers inoculated with the larkspur organism rotted very promptly.

CONTROL MEASURES

According to Dodge,[7] thorough drenching of plants and the adjacent soil with Bordeaux mixture will prevent or check the disease. Studies of control measures in California have been confined to seed treatment. Various concentrations of mercuric bichloride proved ineffective.[8] In field trials in 1937, clean, healthy plants developed from seed that had been treated in hot water at temperatures ranging from 50° to 55° C. for ten minutes. Control plants from nontreated seed showed 18 per cent infection. The results suggest that the organism may be carried in the seed. For practical purposes, it is believed the hot water treatment of seed will give satisfactory control of the disease.

SUMMARY

A bacterial bud and stem rot of rocket larkspur, prevalent in California, has been described.

Except for minor differences, the causal organism agrees rather closely with *Erwinia phytophthora.*

All tested varieties of *Delphinium* were susceptible to infection under greenhouse conditions.

The disease may be controlled by treating the seed for 10 minutes in hot water at 50° to 55° C.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIF.

ALGAL FRUIT SPOT OF ORANGE

J. R. WINSTON[1]

(Accepted for publication January 28, 1938)

The alga, *Cephaleuros (virescens) mycoidea* Karst., attacks a wide range of plants in tropical and subtropical regions. It has been reported at various times on numerous hosts in Florida, including citrus species, of which limbs, twigs, and leaves have been attacked. The disease of citrus caused by this alga rarely, if ever, occurs on the dry sand hills of the interior, but is fairly common on the lowlands along the Florida coast. It is more abundant in the southern part of the State, but even there this alga has not heretofore assumed economic importance. Ruehle, in Plant Disease Reporter, vol. 20, no. 14, August, 1936, reported the disease as being unusually abundant in 1936, and listed the hosts that were found infected. No account of the occurrence of the alga on the fruit of citrus has been located in the literature.

[7] See footnote 2.

[8] The writers found that 1-to-1000 or even 1-to-2000 solutions of $HgCl_2$ (five- and ten-minute treatments) were injurious to larkspur seeds, causing delayed germination and producing very unthrifty plants.

[1] Senior horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

In May, 1937, the writer first saw this hitherto unobserved blemish on the rind of Lue Gim Gong oranges (*Citrus sinensis* Osbeck) then being processed for marketing (Fig. 1). The blemish was a distinctly dark brown to almost black spot, much darker than melanose or exanthema lesions, slightly raised, and with irregular to acutely pointed margins suggestive of lateral root-like developments (Fig. 2). Few, if any, of the blemishes were

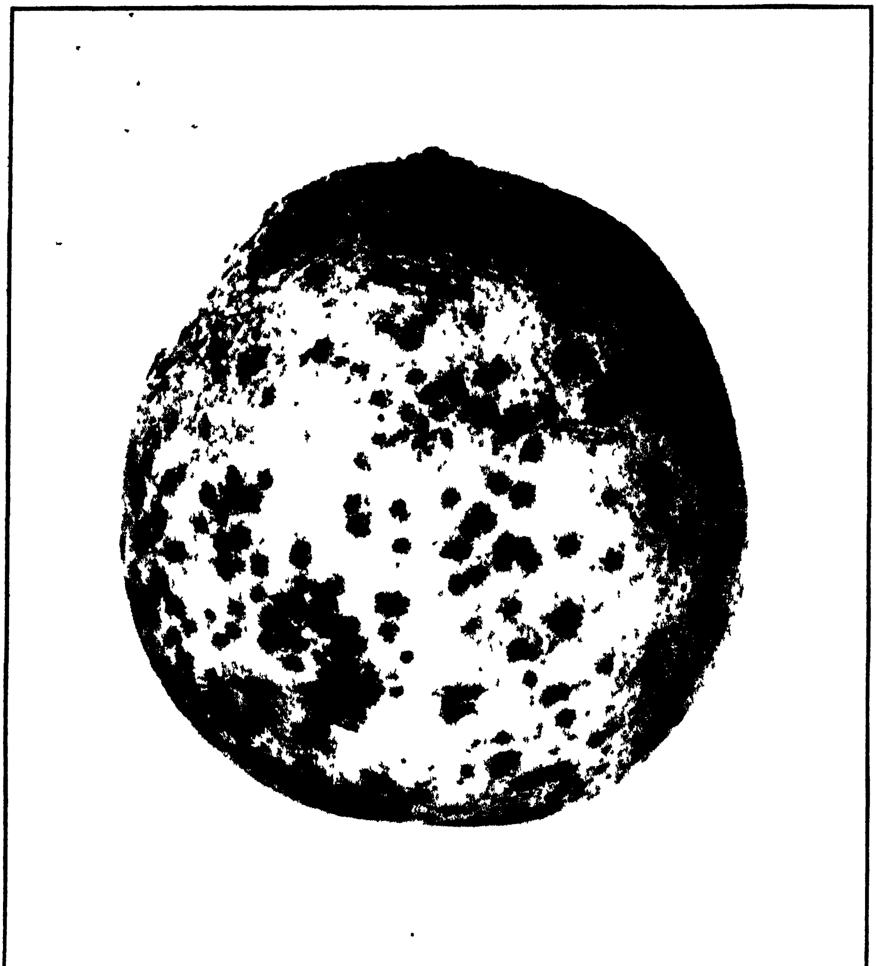

FIG. 1. Lue Gim Gong orange showing spots caused by *Cephaleuros* (*virescens*) *mycoidea* Karst.

as much as 2 mm. in diameter, and most of them were about 1 mm. or slightly less. Free-hand sections through the affected parts showed a slight penetration and a discoloration, rarely extending deeper than 3 or 4 cells into the flavedo. Identification of the causal organism was made by H. R. Fulton and J. A. Stevenson of the Bureau of Plant Industry and typical specimens were deposited in the Bureau herbarium.

FIG 2 Enlarged view of alga spots showing root like appearance.

The infected fruit was produced on 7- to 9-year-old trees in a 400-acre grove located on the edge of the Everglades, about 8 miles southwest of Fort Lauderdale This grove was inspected on June 9, while both mature and very immature fruits were on the trees, and at several other times during the summer and fall Although the disease was found to be rather wide-

spread, it affected but a small percentage of the crop. Liberal samples of fruit from the grove have been held under observation at 80° F., but no evidence has been obtained thus far to suggest that the algal lesions contribute to decay development. Since the Lue Gim Gong oranges harvested in 1937 developed from bloom that opened in the spring of 1936, it is likely that the infection herein reported was a part of the general outbreak reported by Ruehle. Limb and twig infections are not difficult to find, but no infected fruit from 1937 bloom has been found.

The conditions in the grove where the infected fruit was found are worthy of note. The grove is located on drained saw-grass muck land where the muck varies in depth from 5 feet to but a few inches and has a pH ranging from 4.3 to 8.3. The trees are set on ridges with small drainage ditches every 90 feet, emptying eventually into a large drainage canal. Weeds and vines, particularly the cucumber vine, overrun the trees unless mowed frequently and cause the loss of much of the tree foliage, thereby weakening the twigs. It is probable that these conditions favor the development of *Cephaleuros* and render the trees more susceptible to other diseases, as well.

PHYTOPATHOLOGICAL NOTE

Mosaic Resistance in Nicotiana tabacum L.—A breeding program having for its object the production of mosaic-resistant tobacco varieties of types suitable for culture in this country has been in progress for several years. In connection with this project, studies have been made to determine (a) the occurrence of resistance to common tobacco mosaic, and (b) genetic factors affecting resistance.

Tests of 897 seed collections from Mexico and Central and South America, the natural habitat of the species *Nicotiana tabacum,* have yielded 36 lots resistant to mosaic. All came from Colombia, South America. In addition to the variety Ambalema, previously reported by Nolla and Roque,[1] 6 additional distinct named varieties resistant to mosaic were obtained. Despite the fact that mosaic is a common disease throughout the region from which these resistant varieties were obtained, there was no evidence that growers there paid any attention to the mosaic-resistant qualities. It would seem likely that these varieties may be an inheritance from the early and highly developed Indian agriculture that was centered in this area.

Tests with the different mosaic-resistant collections have shown that none are immune. Under suitable conditions they become systemically infected and produce from very slight to rather marked leaf symptoms (Fig. 1). On the basis of leaf symptoms, 3 classes of resistance have been established:

Class 1. None to very faint markings.
" 2. Distinct diffuse spotting.
" 3. Mild systemic mottling.

Studies on the inheritance of resistance showed that resistant lines, de-

[1] Nolla, J. A. B., and Arturo Roque. A variety of tobacco resistant to ordinary tobacco mosaic. Jour. Dept. Agr. P. R. 17: 301–303. 1933.

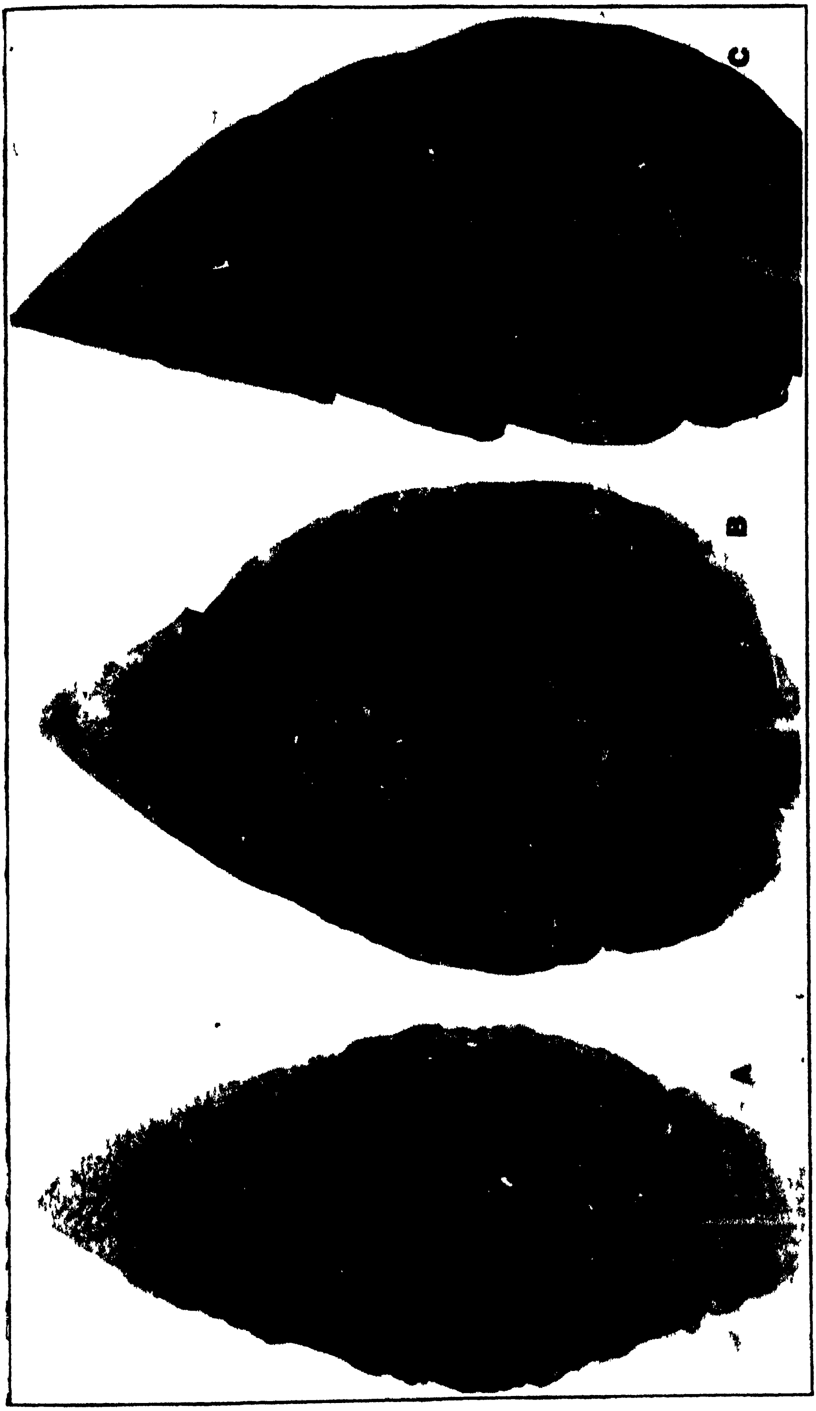

FIG. 1. Effect of degree of resistance on tobacco mosaic symptoms. A. Leaf from a susceptible plant showing characteristic mottling. B. Leaf from a resistant plant having the two Ambalema genes for resistance plus modifiers; the result is the Class 2, diffuse spotting reaction. C. Leaf from a plant having the same resistance genes without the modifiers; result, a Class 1 reaction.

rived from crosses between Ambalema and susceptible domestic varieties, gave, for the most part, a Class 2 reaction. Some of these lines segregated in the F_3 generation, yielding individuals with the Class 3 reaction; which, in turn, produced uniform F_4 populations of this mild-systemic or least-resistant type.

In 1936, tests with both the original F_2 (Ambalema × susceptible variety) and F_2's from resistant selections crossed again with susceptible varieties gave a close approximation to a 15 susceptible to 1 resistant ratio. The total population studied was 8,827 and the value for deviation divided by probable error was 0.76. It was concluded that 2 independent pairs of recessive genes are of major importance in controlling resistance. This verifies a conclusion tentatively reached by Valleau.[2]

As further proof of this conclusion, in 1937 4 first-generation populations, obtained by backcrossing the original F_1's to Ambalema, segregated into a ratio that closely approximated the expected 3 susceptible to 1 resistant. The number of individuals was 2,038 and the value for deviation divided by probable error was 0.50.

However, in 1937, 23 F_2 populations derived from the second backcross of resistant selections to susceptible parent varieties, gave only about two-thirds of the expected number of resistant plants. This deficiency was statistically significant and not a chance variation, since the deviation from a 15:1 ratio was 23.79 times the probable error, and a large population of 27,202 individuals was tested. It is believed that the reduced number of resistant plants was due to the occurrence of many individuals with the Class-3 type of resistance, which, on the basis of symptoms produced on young plants, cannot be separated readily from susceptibles.

We have, then, 3 indications of the existence of genetic factors that modify the expression of the 2 basic-resistance genes; they are:

(1) The decreasing proportion of typical resistant plants in the F_2 after the second backcross to the susceptible parent. This can be explained on the basis of accumulating modifying genes that reduce the resistance from Class 1 or 2 to Class 3.

(2) Resistant F_2 selections, from Ambalema crossed with susceptible varieties, breed true for resistance in the F_3, but may segregate for Classes 1, 2 and 3.

(3) Different degrees of resistance were found in the collections obtained from Colombia. F_1 crosses between Ambalema and different collections falling in Classes 1 and 2 were all resistant, showing that the 2 major recessive genes for resistance were the same in each. Differences are caused by modifiers.

The existence of these modifying genes which reduce mosaic resistance offers a very definite problem in a breeding program that involves frequent backcrosses to the susceptible parent.—E. E. CLAYTON, H. H. SMITH, and H. H. FOSTER, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[2] Kentucky Agr. Exp. Station, Ann. Rpt. (1936) 49: 28–29. 1937.

BOOK REVIEW

HEALD, FREDERICK DEFOREST. *Introduction to Plant Pathology.* 1st Ed. 579 pp. McGraw-Hill Book Company, New York and London. 1937. $4.00.

An event of great importance to teachers of plant pathology is the publication of a new book, *Introduction to Plant Pathology*, by Dr. F. D. Heald, which should prove as satisfactory a teaching text as his *Manual of Plant Diseases* is as a reference work. The pages of the new book are replete with valuable information expressed in such a clear, forceful style that it might well be a model for biological text books of the future.

The *Introduction to Plant Pathology* retains much of the material found in his larger *Manual of Plant Diseases*, but it is rearranged and presented in such an order that the student, after being introduced to the study of plant diseases in a general way, is led directly into the section dealing with maladies caused by the higher fungi. Teachers in general will recognize the pedagogical value in the student's having an early introduction to those diseases of greatest interest and importance. A valuable, though rather abbreviated, section on phytopathological methods very properly comes at the end of the text, as few students would be concerned with the technique of investigational work before they had studied specific diseases.

The entire text is evidently the result of careful planning. The diseases discussed are without exception those that would be of interest and importance throughout the United States and Canada. The author employs the sound pedagogical system of describing fully the characteristics of a few typical diseases caused by members of the various important groups of the fungi, bacteria, and other pathogenic agencies. The representative of each group was aptly selected because of its widespread importance, its historical interest, or its value in illustrating some particularly important feature.

While Heald uses the mycological rather than the host basis for arranging his material, he changes the arrangement usually employed by placing the bacterial diseases after those caused by the true fungi. The characteristics of the different groups of the fungi are presented in considerable detail at the beginning of the various chapters. Had some type of descriptive key to the various groups of colorless thallophytes been included in one of the introductory chapters for general reference, the student might get an even broader perspective and a better grasp of this phase of the subject matter than he can with its present treatment.

There are a few cases of inconsistency, especially in terminology, that might have been avoided. It is unfortunate, for instance, that the author, while treating of physiologic specialization, did not use the term "physiologic races" exclusively, although it may be that he distinguishes in his own thought between "physiologic races," "physiologic strains," and "biologic strains." Likewise, if he had used only the Bergey (*et al.*) system of classifying bacteria, he would have been saved the embarrassment of having at times to employ the nomenclature of that system, while he, himself, sponsors that of Migula.

The viroses and problems associated with them are treated comprehensively. It would be difficult to improve upon the organization of this important section of the text, the entire subject of plant viroses being covered in a condensed treatment to better advantage than in any general text known to the reviewer.

The nonparasitic diseases are discussed thoroughly, possibly at the expense of some of the parasitic troubles of greater importance. It is noticeable, at least, that the author does not need to cite a list of supplementary physiologic diseases not discussed in the text.

The chapter on the relation of fungi and bacteria to human affairs contains a great deal of useful information. It is material, however, that probably could be more suitably presented in text books of general botany or microbiology than in one of plant pathology. If the pages thus employed had been used in presenting a good glossary, the text might be even more useful to the beginning or advanced student in plant pathology than it is now.

Like other text books and, indeed, like scientific papers in general in the field of plant pathology, the "Introduction" does not give a complete picture of the signs and symptoms of the various diseases from a developmental basis. The characteristics that mark the final stage of the diseased condition are usually adequate, but there seldom is a description of the early stages with an account of the changes in color, elevation, and other features that occur as the disease develops. The incorporation of descriptions of such a type probably would be one of the greatest contributions that Dr. Heald could make to a later edition, when he thinks that the present one needs revising.

Those errors that occur, including those of omission, however, are relatively few and can easily be explained by the fact that the author probably endeavored to keep the text in reach of the undergraduate student, both in a financial and pedagogical sense.

It should be emphasized that plant pathologists, especially those engaged in teaching, are deeply indebted to Dr. Heald for a modern, general textbook on plant diseases, which, in the reviewer's opinion, is the best that has ever been published for the general college student, county agent, and extension specialist. Its appearance should be regarded as one of the prominent landmarks in the history of plant pathology.—H. H. HAYMAKER, Kansas State College, Manhattan, Kansas.

REPORT OF THE TWENTY-NINTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1937 INDIANAPOLIS MEETING

A very successful and well-attended meeting was held at Indianapolis, Indiana, December 27 to 30, 1937. H. W. Anderson, University of Illinois, was elected president for 1938; R. W. Goss, University of Nebraska, vice-president; and J. J. Christensen, University of Minnesota, councilor. R. S. Kirby, Pennsylvania State College, was appointed secretary *vice* G. H. Coons, resigned; H. A. Edson, U. S. Department of Agriculture, was reappointed treasurer and business manager of PHYTOPATHOLOGY; and H. B. Humphrey, U. S. Department of Agriculture, was reappointed editor in chief of PHYTOPATHOLOGY.

About 200 members were present, and the annual dinner was enjoyed by approximately 300. The election of 124 new members brought the active enrollment, for the first time, above the thousand mark, to 1,053. The scientific program included 126 prepared papers. Special conferences were held on problems relating to plant quarantines and seed-borne diseases and on extension methods in plant-disease control and their results. Group attack on important plant disease problems was advanced in special meetings held by members of the Tobacco Disease Council and Cotton Disease Council. Arrangements were made for a symposium program in connection with the Ottawa meeting in June 1938.

The necessity for closest cooperation between different groups of scientists in solving important problems of agriculture was recognized in a joint symposium at which entomologists and plant pathologists discussed the manifold relationships of insects in the spread of many diseases of fruits, truck crops, grain crops, trees, etc. Joint sessions were held with Section G, A. A. A. S., the Mycological Society of America, the Potato Association of America, and the Floriculture Section of the American Society for Horticultural Science.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1938

Officers:

H. W. ANDERSON, President (1 yr.), University of Illinois, Urbana, Ill.

R. W. GOSS, Vice-President (1 yr.), University of Nebraska, Lincoln, Nebr.

R. S. KIRBY, Secretary (3 yrs. Term expires 1940), Pennsylvania State College, State College, Pa.

H. A. EDSON, Treasurer and Business Manager of PHYTOPATHOLOGY (3 yrs. Term expires 1940), U. S. Department of Agriculture, Washington, D. C.

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R. S. KIRBY, Advertising Manager of PHYTOPATHOLOGY (1 yr.), Pennsylvania State College, State College, Pa.

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G. W. KEITT (Term expires 1938), University of Wisconsin, Madison, Wis.

CHAS. CHUPP (Term expires 1938), Cornell University, Ithaca, N. Y.

J. J. CHRISTENSEN (Term expires 1939), University of Minnesota, St. Paul, Minn.

A. N. BROOKS (for the Southern Div.), Florida Experiment Station, Lakeland, Fla.

J. W. HOTSON (for the Pacific Div.), University of Washington, Seattle, Wash.

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A. A. A. S. Council (1 yr.), W. D. Valleau, S. A. Wingard.

Elector Group V, Division of Biology and Agriculture, National Research Council (3 yrs.), E. C. Stakman (H. P. Barss, alternate). (Terms expire 1940.)

Board of Governors, Crop Protection Institute (3 yrs.), K. J. Kadow (Term expires 1939), C. R. Orton (Term expires 1938), J. G. Horsfall (Term expires 1940).

Tropical Research Foundation (5 yrs.), L. R. Jones (Term expires 1940).

International Union of Biological Sciences, A. G. Newhall.

Board of Editors, American Journal of Botany, G. W. Keitt (3 yrs. Term expires 1940).

Union of American Biological Societies [*and Biological Abstracts*], Editor in Chief (H. B. Humphrey) and Secretary (R. S. Kirby) *ex officiis*, G. W. Keitt, H. A. Edson, D. Reddick, H. P. Barss, Chm.

American Type Culture Collection, C. L. Shear (5 yrs. Term expires 1942).

Standing Committees:

Regulatory Work and Foreign Plant Diseases, C. R. Orton, Chm., H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands, J. F. Adams, E. L. Chambers.

Extension Work, Luther Shaw, Chm., Chas. Chupp, R. J. Haskell, A. L. Pierstorff, R. S. Kirby, E. C. Stakman, G. W. Keitt, W. B. Tisdale, I. L. Conners.

Coordination in Seed Treatment Research, C. S. Reddy, Chm., W. E. Brentzel, M. B. Moore, H. A. Rodenhiser.

Phytopathological Classics, H. H. Whetzel, Manager; H. B. Humphrey, Editor.

Necrology, A. G. Johnson, Chm., M. B. Waite.

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Donations and Legacies, F. C. Meier, Chm., E. C. Stakman, N. E. Stevens, J. G. Brown, N. J. Giddings.

New Memberships and Subscriptions, A. J. Riker, Chm., R. M. Lindgren, J. C. Carter, B. A. Rudolph, K. J. Kadow, R. S. Kirby (*ex officio*).

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Elections, E. E. Honey, H. W. Dye.

Resolutions, E. Carsner, C. E. Owens, C. J. Nusbaum.

Committee on Arrangements, Ottawa Meeting, H. T. Güssow, Chm., F. L. Drayton, H. B. Humphrey.

REPORTS OF OFFICERS, REPRESENTATIVES AND COMMITTEES FOR 1937

Report of the Secretary. The Society year 1937 opened with 965 members and closed with 1053, a net gain of 88. At the Indianapolis meeting 124 new members were elected. Eight former members were restored to the active roll during the year. The Society lost 44 members, 14 by resignation, 5 by death and 25 by suspension for non-payment of dues. Of the full membership, 120 are paid-up life members and 42 are paying $10.00 per year toward life membership.

During his three-year term of service the retiring secretary has enjoyed such a full measure of effective cooperation from the officers, committees, and general membership of the Society that his tasks have been made pleasant. He also has appreciated the efficient services of Mrs. Fred C. Meier in keeping the records and the help of Miss Ruth A. Smith in the correspondence and office work.

HOWARD P. BARSS.

Report of the Treasurer. Statement of accounts for the year ending November 30, 1937.

Receipts:

Balance from 1936			$2,391.54
Annual dues:			
1935	5.00		
1936	29.34	($ 1.00 life)	
1937	2,670.56	(270.10 life)	
1938	2,017.81	(300.66 life)	

1939	13.82 (5.00 life)		
1940	1.75	$4,738.28	
Voluntary dues			
1937	$ 10.00		
1938	17.50	27.50	
Publication of abstracts, 1936		129.70	
Items for other accounts included in checks for dues:			
Lyman Fund	$ 5.00		
Publ. papers	31.50		
Subscriptions	1.00		
Sales	7.60	45.10	
To replace check returned by bank		5.00	
Bank error corrected		0.25	
Total receipts			4,945.83
			7,337.37
Expenditures:			
Member subscriptions transferred to PHYTOPATHOLOGY			
1935	$ 4.00		
1936	26.64		
1937	3,433.08	3,463.72	
Transferred to Sinking Fund (Building and Loan)		290.66	
Publication of Society material in PHYTOPATHOLOGY (January to June, 1937)		229.33	
Publication of abstracts		114.58	
Proofreading "Classics"		15.60	
Secretarial work for Secretary and Treasurer		287.63	
Printing and mimeographing		154.15	
Stamps and envelopes		88.35	
Supplies		8.35	
Loss on A. P. S. dinner, Atlantic City		1.60	
Contribution to Biologists' Smoker		10.00	
Binding 5 volumes official set of PHYTOPATHOLOGY		8.75	
Embossed parchment		8.23	
Membership committee, expenses		25.70	
Transferred to PHYTOPATHOLOGY for items in checks for dues			
Publ. of papers	31.50		
Subscriptions	1.00		
Sales	7.60	40.10	
Transferred to PHYTOPATHOLOGY for voluntary dues, 1937		10.00	
Telegram		1.81	
Transferred to Lyman Fund		5.00	
Check returned by bank		5.00	
Collection charges on checks		4.75	
Bank error		0.25	
Total expenditures			4,773.56
Balance on hand			2,563.81
			7,337.37

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, is obtained by deducting $5.00 from each $10.00 life-membership installment. This fund totaled $9,036.00 at the close of 1936. During the 11 months January to November, 1937, it has increased to $9,281.66 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection ($2,000 at 6%, $1,000 at 5½%, $500 at 5%)	$3,500.00
Invested with the following building and loan associations:	
Arlington & Fairfax Bldg. and Loan, 5%	1,000.00
Columbia Permanent Bldg. Ass'n, 4½%	500.00
District Bldg. and Loan Ass'n, 4%	1,000.00
National Permanent Bldg. Ass'n, 5%	525.40
Northwestern Savings and Loan Ass'n, 5%	1,000.00
Perpetual Bldg. Ass'n, 4½%	1,022.50
Prudential Bldg. Ass'n, 4%	781.66
	9,329.56
Less interest payable to PHYTOPATHOLOGY (National Permanent and Perpetual)	47.90
	9,281.66

The Lyman Memorial Fund for the permanent endowment of PHYTOPATHOLOGY, obtained from voluntary contributions, totaled $2,608.81 at the close of 1936. During the 11 months, January to November, 1937, this fund has increased to $2,709.81.

Invested with the Brookland Building Ass'n, 4%	$2,762.76
Less interest payable to PHYTOPATHOLOGY	52.95
	2,709.81

H. A. EDSON.

Report of the Business Manager of Phytopathology. At the end of 1936 there were 592 subscriptions to PHYTOPATHOLOGY including 6 complimentary. During 1937 there were 33 cancellations, and 27 suspensions for non payment (including our total subscription list for Spain), a loss of 60, but with 82 new paid and one new complimentary subscription, the net gain is 23, increasing the list at the close of 1937 to 615. Of these 185 are domestic and 430 are foreign. Japan leads with 67, U. S. S. R. has 54, England 37, Canada, 26, Argentina and China 23 each and Germany 22. Sixty-two other countries or geographical units receive from one to 19 copies each.

Statement of accounts for the year ending November 30, 1937.

Receipts:

Balance from 1936			$ 1,407.49
Subscriptions:			
1936	$ 65.60		
1937	3,173.35		
1938	343.69		
1939	11.35		
1940–1943 (5.85 each)	23.40	$3,617.39	
Member subscriptions, 1935, 1936		30.64	
Voluntary dues, 1937		10.00	
Member subscriptions, 1937		3,433.08	
Sales of back numbers		423.80	
Advertising:			
1936	$ 229.87		
1937	958.48	1,188.35	

Interest on Sinking Fund:				
First mortgage notes		263.06		
Building and loan		143.31[1]	406.37	
Interest on Lyman Fund			51.60[2]	
Publication of papers, 1936			150.50	
From A. P. S. for publication of Society material			229.33	
Allowance on reprints by printer			51.93	
First mortgage note paid in full			1,000.00	
Total receipts				10,592.99
				12,000.48

Expenditures:

Printing and distributing PHYTOPATHOLOGY:				
Vol. XXVI, No. 12 (containing Index)		$ 627.52		
Vol. XXVII, No. 1	$692.05			
No. 2	566.99			
No. 3	667.54			
No. 4	372.97			
No. 5	395.61			
No. 6	462.07			
No. 7	412.25			
No. 8	472.66			
No. 9	477.67			
No. 10	472.33			
No. 11	629.78	5,621.92		
Postage		533.99	$6,783.43	
Secretarial work for Editor in Chief			353.86	
Secretarial work for Business Manager			155.06	
Secretarial work and expenses of Advertising Manager			32.85	
Commission, Advertising Manager			98.96	
Stamps and envelopes, Bus. Mgr. and Editor in Chief			84.18	
Supplies			3.30	
Printing			36.00	
Reinvestment, Sinking Fund			1,000.00	
Refund subscription			5.85	
Refund, sales			2.25	
Charge for collection of check			0.15	
Total expenditures				$ 8,555.89
Balance on hand				3,444.59
				12,000.48

H. A. EDSON.

Report of the Auditing Committee for the Year Ending November 30, 1937. The books of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY have been examined, together with the present investments of the Sinking Fund and the Lyman Memorial Fund. The accounts have been found correct and the books in very excellent order.

December 20, 1937. J. E. KOTILA, CARL HARTLEY.

[1] $47.90 of matured interest has been left invested until needed.
[2] $52.95 of matured interest has been left invested until needed.

Report of the Advertising Manager. The 1937 advertising receipts of $1,182.05 from PHYTOPATHOLOGY are the largest for any of the past six years. The 1937 receipts were $201.50 greater than those of 1936, or a percentage increase of 20.4.

The total number of advertisements run in 1937 was 204. Of these, 153, or 75 per cent, were revenue advertisements, 32 were full-page, 49 were half-page, 56 were quarter-page, and 16 were one-eighth page advertisements.

The increase of $201.50 in the amount of receipts from advertising was due to better business conditions and to aid of members in helping the advertising manager contact new sources for advertising.

Fifty-one advertisements occupying 37.5 pages were non revenue-producing. These consisted of exchange advertisements with other journals, space occupied by the Directory of Advertisers, announcements of meetings, and Phytopathological Classics.

During 1937, 22 concerns purchased advertising space in PHYTOPATHOLOGY.

R. S. KIRBY.

Report of the Editor in Chief. PHYTOPATHOLOGY, Volume 27, 1937, comprises, exclusive of the index, 1,185 pages (25 pages more than in Volume 26) of printed matter, including illustrations, classified as follows: Eighty eight articles, 34 phytopathological notes, 2 reports of regional and other meetings, 5 book reviews, 93 abstracts (4 by title only), 177 text figures, 3 plates, and 2 frontispieces. From Jan. 1 to Dec. 31, 1937, 108 manuscripts of articles, notes, reports, and book reviews were submitted for publication in PHYTOPATHOLOGY. Of this number 2 were not accepted, 1 was recalled by the author, and 33 were returned to their authors for more or less complete revision and condensation. Of those manuscripts received in 1936, but too late for publication in Volume 26, 12 were returned to their authors for revision. The index for Volume 27 was published in the December number.

Manuscripts submitted during the current year have shown an appreciable improvement in organization of subject matter, conciseness, clarity, and picturization, and also in their value as contributions to our accumulating knowledge of plant pathology. The journal and, incidentally, the Society have profited measurably through the editorial assistance of Dr. F. V. Rand, who kindly prepared the index for Volume 27. The Society also is indebted to The Science Press Printing Company for its share in enabling us to publish a journal of distinction from the standpoint of printers' craftsmanship.

H. B. HUMPHREY.

Report of the Manager of Phytopathological Classics for the fiscal year December 15, 1936, to December 15, 1937.

Classics No. 1:	On hand 12-15-36	220	
	Sold during year	45	
	On hand 12-15-37		175
Classics No. 2:	On hand 12-15-36	419	
	Sold during year	41	
	On hand 12-15-37		378
Classics No. 3:	On hand 12-15-36	545	
	Sold during year	57	
	On hand 12-15-37		488
Classics No. 4:	On hand 12-15-36	609	
	Sold during year	52	
	On hand 12-15-37		557

Classics No. 5: Printed August, 1937		1091	
	C. R. ORTON,		
Sold	180		
Gratis	12		
Disposed of during year		192	
On hand 12–15–37			899

Cash balance on hand Dec. 15, 1936	$418.09	
Receipts during year	277.18	
Total cash income		$695.27
Expenditures:		
Printing Classics No. 5	$601.76	
Advertising	6.48	
Postage, etc.	45.26	
Stationery and supplies	17.66	
Total cash expenditures		$671.16
Balance on hand 12–15–37		$ 24.11
Balances due on account 12–15–37		$ 41.50

H. H. WHETZEL.

Report of the Committee on Necrology. During the calendar year 1937, there have been five deaths of members as follows:

Dr. J. J. Davis, February 26.
Mr. Stacy O. Hawkins, July 10.
Dr. G. P. Clinton, August 13.
Dr. F. Sattler, September 12.
Dr. J. J. Taubenhaus, December 13.

A. G. JOHNSON,
M. B. WAITE.

Following the reading of the necrology report the members present stood for a moment in silence in honor of their departed colleagues.

Report of the Representative for Group V in the Division of Biology and Agriculture of the National Research Council. The three-year term of your representative ended July 1, 1937. The representative for Group V (bacteriology, plant pathology, mycology) for the present three-year term is Dr. E. C. Fred, Agricultural Bacteriologist, University of Wisconsin. Of interest to this Society among the activities of the Division during the past fiscal year was the establishment of two important interdivisional committees, which include other groups of sciences embraced within the National Research Council with the Division of Biology and Agriculture. The first is a Committee on the Genetics of Pathogenic Organisms, E. C. Stakman, Chairman. This committee is organizing a symposium on this subject in relation to human welfare for the Ottawa meeting in June 1938. The other is the Committee on Aerobiology, Fred C. Meier chairman. This committee is concerned with the aerial dissemination of microorganisms and such other disease-producing agents as viruses and allergens affecting the health of animal and plant life as well as that of humanity. The object of these committees is to facilitate the bringing together of representatives of various scientific groups in these fields for mutual assistance in order to promote the most rapid advancement of important knowledge through better-coordinated efforts. E. C. Stakman was appointed vice-chairman of the Division of Biology and Agriculture for 1937–38. Howard P. Barss was appointed representative of the National Research Council on the Board of Governors of the Crop Protection Institute for the three-year period beginning July 1, 1937.

HOWARD P. BARSS (R. F. POOLE, Alternate).

Report of the Representatives on the Board of Governors of the Crop Protection Institute. The Institute is organized as a non-profit organization. Its purpose is to make available the services of technically trained men under competent direction for research relating chiefly to the effectiveness of various types of materials or apparatus for the control of plant diseases and insect pests, or similar types of problems, for the benefit of commercial manufacturers needing dependable scientific assistance in these fields and for the ultimate benefit of agriculture, as well as for the advancement of science. The Board of Governors is composed exclusively of representatives elected by The American Phytopathological Society, The American Association of Economic Entomologists, the Association of Official Agricultural Chemists, and the National Research Council. Dr. W. C. O'Kane was again reelected chairman of the Board for 1938.

An active program of research was continued during the year under the auspices of the Institute, with nine major projects in progress on November 15, 1937, in addition to which a considerable amount of preliminary or exploratory work was under way to determine whether certain materials appeared to have sufficient merit to warrant the establishment of formal research projects for their more thorough study.

The Atlantic Refining Co., National Aniline and Chemical Co., Dow Chemical Co., Röhm and Haas Co., Standard Chemical Products, Inc., and General Chemical Co. are contributing to the support of such major projects. Exploratory work was done on the products of some 20 other firms. The work was conducted in various States, including New Hampshire, Connecticut, New York, Delaware, Maryland, Virginia, Indiana, Iowa, Louisiana, and California.

The following bulletins were published in the Institute series in 1937:

No. 61, "Contact Insecticidal Properties of Various Derivatives of Cyclohexylamine," by C. W. Kearns and W. P. Flint.

No. 62, "Evaluation of Cuprous Oxides Recommended as Seed Treatments for the Control of Damping-Off," by H. W. Anderson, K. J. Kadow and S. L. Hopperstead.

No. 63, "Copper Sulfate as a Plant Nutrient and Soil Amendment," by W. L. Churchman, M. M. Manns and T. F. Manns.

W. H. MARTIN,
J. F. ADAMS,
C. R. ORTON.

Report of the Committee on Publication Problems. Following favorable action by the Society at the Atlantic City meeting on proposals submitted by your Committee on Publication Problems, the Editorial Board with the strong backing of the membership of the Society put into operation a more vigorous editorial policy looking toward raising the standards of subject-matter quality and maximum brevity and conciseness consistent with scientific usefulness. Very satisfactory have been the results of the combined efforts of our contributing members and our editors. The number of articles and the quality have increased during the year without demanding increased space. As a result the journal has been able to publish all papers without delay other than that required for editing, necessary revision, and printing.

The Editor in Chief and Business Manager were authorized by the Society at the latest meeting to effect such economies as they deemed wise in the structure of PHYTOPATHOLOGY. Some space-saving measures have been adopted without requiring any essential change in format.

At the close of the year the Journal finds that its income has been adequate. There is every expectation that this will continue and will permit prompt publication of all acceptable contributions during the year ahead. The additional memberships obtained by the Membership Committee, appointed by the Society on recommendation of this Committee, are helping to provide the needed additional support.

In view of the effectiveness of the steps already taken by the Society and the machinery set up by it for still further adjustment, if necessary, in future, the Committee considers its functions completed and requests that it be discharged.

In closing its work, the Committee desires to urge that the members of the Society continue their vigorous support of the Editorial Board and Management of PHYTOPATHOLOGY in maintaining the high standards of quality, conciseness, and economy in publication that they are endeavoring to promote.

H. B. HUMPHREY, H. A. EDSON, R. S. KIRBY, E. C. STAKMAN, N. E. STEVENS, M. W. GARDNER, R. F. POOLE, L. M. MASSEY, L. R. JONES, F. L. DRAYTON, G. W. KEITT, HOWARD P. BARSS, Chairman.

Report of Committee on Regulatory Work and Foreign Plant Diseases. A general meeting of the Society was called in the evening of December 27 to hear reports and discuss phases of the foreign plant-disease and quarantine problem.

A progress report of the subcommittee, prepared by Messrs. Munn, Scott, and Brown, on seed-borne parasites, was presented by the chairman of the meeting. Plans for strengthening the requirements for disease examination of seed stocks passed in international commerce were discussed at the Congress of International Seed Testing Associations in 1937 and are underway in cooperation with committees from other nations. In August, 1937, the subcommittee secured the passage of the following resolution at the meeting in Washington of the Association of Official Seed Analysts. "Resolved, that the Association recommend to all laboratories that in the future they recognize the significance of the plant disease carrying possibilities of seed stocks, and that in providing for the personnel of their staff, and more particularly in arranging report blanks or forms, that provision be made to care for the reporting upon the sanitary condition or health of the seed stock being tested, along with the other factors now being determined." The subcommittee is mindful of proposed federal legislation to regulate the sale and transport of seed stocks in interstate commerce that at present, does not provide for standards regarding the presence of harmful plant diseases.

The following practical suggestions were made by the subcommittee:

1. Steps now being made to appraise the disease-distributing possibilities of the better seed stocks through certification or otherwise should be fully supported by every agency.
2. Seed-testing and control laboratories should be fully supported in their attempts to do the practical determinations possible and then go farther by undertaking definite investigations of the situation as regards the sanitary conditions of the seed stocks being used each season.
3. Every attempt should be made to stop, or at least very materially limit or curb, the movement, sale, or use under the caption of "seed" of great volumes of grains of nondescript origin, variety and sanitary condition now being diverted out of commercial channels and shipped more or less promiscuously over the country, sooner or later to take on the more dignified name of "seed stocks." Our committee believes that the name "seed," in its agricultural sense, should mean something definite. It should mean more than that it will produce a living plant; it should also mean that it will produce a healthy plant.
4. In preparing seed-control legislation and facilities for seed-stock control, there should be given due consideration to the possibilities of restricting, curtailing, or even prohibiting with utmost promptness the movement of definitely disease-infested seed stocks in the same manner as that of weed-seed-contaminated seed stocks, insofar as practicable. Seed-stock control should include whatever is known to be practical in the realm of seed treatments.

The report of the subcommittee was accepted with appreciation and a request that they continue their efforts and report at the next meeting.

At the suggestion of President Keitt, a motion was passed that a subcommittee be appointed to study the subject of State regulatory work and report at the next meeting. Mr. E. L. Chambers was appointed chairman of this subcommittee and Dr. J. F. Adams a member.

A motion was passed that the Council of the Society consider the appointment of a delegate to represent the Society, if called upon to do so, in connection with the work of a committee appointed by the League of Nations to consider matters relating to plant-quarantine regulations.

A motion was passed that the Council of the Society designate a member to attend any conferences called to consider possible Congressional action to govern the entry of pathogens or pests into this country.

Dr. Adams reported the activities of the National Plant Board for 1937, covering (1) nursery inspection and certification, (2) pest emergency, (3) azalea leaf spot, (4) potato wart, (5) standardization of state quarantines, and (6) national weed control.

C. R. ORTON.

Report of the Representative of the Society with the International Union of Biological Sciences. The next meeting of the Union will be held in Stockholm in July, 1940. The Subsection for Phytopathology is in the process of formation now and will be fully organized before the next convention. The officers are: President, Donald Reddick; Secretary, Johanna Westerdijk. The exact procedure to be employed in drafting international legislation is not yet determined, but it is not likely to differ radically from that employed heretofore by the nomenclature committee of the international botanical congresses. Any society, group, or individual doubtless will have the privilege of presenting subjects for consideration and legislation. Presumably, these proposals will have to be distributed in print at least six months before the convention. February, 1940, would thus be the latest date for announcing proposals; but nothing would prevent printing such announcements a year or more in advance.

Three items are pending from the Amsterdam congress.

1. *English, an International Language.* Violent opposition voiced at Amsterdam was based in part on misunderstanding of the proposal. (The proposal was not distributed in advance of the meeting.) It is noticeable, however, that without any legislation at all, summaries in English of papers in other languages are appearing with greater frequency. Our Society is not called upon to support this proposal with any vigor, but we might well pledge cooperation through an endeavor to make our own writings so clear and simple that they can be read with ease rather than with difficulty. Aside from technical words it is likely that a vocabulary of one thousand other words is ample for recording our observations. Most of the papers we publish are documents, not literary productions.

2. *The Nomenclature of Immunology.* A series of definitions was proposed at Amsterdam which was conspicuous because of the lack of a definition for the word immune, itself. Our Society should appoint a committee to study this proposal and make recommendations. Our delegate to Stockholm should be able to present a positive position in this matter.

3. *International Committee for Description and Nomenclature of Plant Viruses.* This committee was formalized at Cambridge and presented a report of progress at Amsterdam. Mimeographed copies of the report were distributed. Although the report is "incomplete" and "preliminary," distribution of copies of it to our members might serve a very useful purpose.

A variety of subjects might well be considered by special committees of the Society with the idea that proposals should be formulated for consideration and international concord.

1. *Word Usage.* Surely, plant pathologists throughout the world should use certain words in a technical sense and with just one definition. This is not being done now and the differences in definition are not confined to the literature of immunology. The proposed committee for study of the nomenclature of immunology might be authorized to broaden the scope of its studies to include the dozen or two dozen words the exact international usage of which would be most helpful in recording our findings.

2. *The Eriksson Institute.* It would be particularly appropriate if a proposal could be made at Stockholm that would make real the international institute of plant pathology

of which Doctor Eriksson used to speak and write on every possible occasion. The Subsection Phytopathology of the Union is, in fact, such an institute. Its professors are the plant pathologists of the world. Its laboratories are the existing laboratories of plant pathology. Its important need is a paid secretary who can give time to the business of coordination of effort, and who will be informed about sources of funds that can be properly applied to the business of the institute.

3. *Naming or Numbering Biotypes, Physiological Races, etc.* A committee of this Society [Humphrey, Stakman, Reddick] has been appointed to study this problem and, if possible, to draft a procedure that the Society may be willing to support at Stockholm.

DONALD REDDICK.

Report of the Resolution Committee. 1. RESOLVED that The American Phytopathological Society express its appreciation to A.A.A.S. committees responsible for the arrangements that have contributed so effectively to the success of the 1937 meeting in Indianapolis.

2. RESOLVED that The American Phytopathological Society convey to the management of the Severin Hotel gratitude for the courteous and efficient service extended to the members attending the 29th annual meeting.

3. Recognizing the splendid condition of our Society and the devoted and time-consuming efforts of our officers and permanent committee members in effecting gratifying advancement:

BE IT RESOLVED, that we express our deep appreciation to them in behalf of the Society.

AND BE IT FURTHER RESOLVED that we pledge ourselves, as members, to cooperate in every possible way with our officers and committees throughout the coming year.

4. RESOLVED that on behalf of The American Phytopathological Society it gives us pleasure to transmit to the Canadian Government and our Canadian colleagues grateful appreciation for the very cordial invitation to the meeting next summer in Ottawa.

5. RESOLVED that the members of The American Phytopathological Society attending the 29th Annual Meeting extend thanks to the entertainment committee composed of Ralph M. Caldwell and Neil E. Stevens for the pleasing program especially the beautiful and artistically rendered songs of Stephen C. Foster, given by the Negro quartette at our annual dinner.

E. CARSNER, C. E. OWENS, C. J. NUSBAUM.

ACTION BY THE SOCIETY AT THE 1937 INDIANAPOLIS MEETING

Elections and Appointments. The appointments made, as provided by the constitution, by the President or the Council since the previous meeting were approved by Society vote in business session. The ballots for the election of officers were unsealed and tallied by the Election Committee. The results were canvassd by the Council and announced to the Society. The names of those elected and appointed appear earlier in this report in the list of "Officers, Representatives, and Committees." Without dissent, 124 new applicants were elected to membership.

Members of the Editorial Board of PHYTOPATHOLOGY elected by the Society on nomination of the Council after consultation with the Editor in chief, H. B. Humphrey, are: Editors (3 yrs. Terms expire 1940), Wm. W. Diehl, C. E. Owens, H. M. Quanjer; Associate Editors (3 yrs. Terms expire 1940), Thorvaldur Johnson, A. J. Riker, W. J. Zaumeyer, Geo. F. Weber.

Following the announcement of the election of G. H. Coons as Secretary, Dr. Coons stated that, although he was appreciative of the honor, the burden of official duties would make it impossible for him to serve the Society in that capacity. This resignation made it incumbent on the 1938 Council to fill the secretaryship, which was done later by the appointment of R. S. Kirby.

Reports of Officers, Representatives, and Committees. The reports for the year 1937, as presented on previous pages, were individually read and accepted by vote of the

Society in business session with the exception of the report of the Committee on Regulatory Work and Foreign Plant Diseases, which was presented more in detail before the conference held under the Committee's auspices Monday evening, December 27.

Amendment to the Constitution on Terms of Office of Division Councilors. After due notice sent to all members early in October, 1937, the members present at the business meeting December 27, 1937, passed without dissent, an amendment to Article V of the Constitution, whereby the last sentence of the second paragraph is changed to read as follows:

> "The term of service of a Council member from a Division shall commence immediately following his election and shall continue until his successor is elected."

Recommendation of Change in Constitution to Provide for Council Appointment of Secretary of the Society. During the year President Keitt appointed a committee (Coons, Chm., Barss, Gardner, Güssow, Reddick, Stakman, with the President *ex officio*) to study sentiment with respect to the present system of electing officers. The Committee reported to the Council that, in general, no change in the present system was favored, except in reference to the secretaryship. After due consideration the Council recommended that the Society be given opportunity to vote at the next annual meeting on an amendment placing upon the Council the duty of appointing the secretary for a term of three years, subject to the usual ratification by the Society.

Subcommittee on State Regulatory Work. On recommendation of the Committee on Foreign Plant Diseases, endorsed by the Council, the Society voted to authorize the formation of a subcommittee on state regulatory work to consider and work out means whereby the Society can be of greater service to those engaged in this important phase of agricultural protection. On Council recommendation the name of the main committee was changed to Committee on Regulatory Work and Foreign Plant Diseases.

Representatives on the Union of American Biological Societies. On Council recommendation the Society voted to discontinue the former Biological Abstracts Committee and to place its functions on the expanded committee representing the Society with the Union of American Biological Societies. At the same time the latter Committee was authorized to approach the membership of the Society in the interest of establishing Biological Abstracts on a permanent, effective basis.

Program Committee. It was voted to lay on the table until the next annual meeting a change in the Standing Rules, Section 4, relative to the constitution and duties of the committee in charge of the programs for annual meetings, which had been recommended by the Council for consideration.

November 10 Set as Latest Date for Receipt of Abstracts of Papers for the Annual Meeting. On Council Recommendation the Society voted without dissent to amend the Standing Rules so as to set November 10 instead of November 15 as the latest date for receipt of abstracts of papers submitted for the annual meetings in order to allow more time for correspondence with authors.

The A.A.A.S. Prize. On Council recommendation the Society voted that the Abstracts Committee shall call to the attention of the Council any papers that might be given consideration in connection with the annual A.A.A.S. Prize.

Place of Next Annual Meeting. On Council recommendation the Society voted to leave to the decision of the Council the place of the next annual meeting.

Representative of the Society in Conferences on Possible Congressional Action Relating to Pathogenic Organisms. On Council recommendation the Society voted to authorize President Keitt and Secretary Barss, after conference with Chairman Orton of the Committee on Regulatory Work and Foreign Plant Diseases, to appoint a delegate to represent the Society at any conferences called in Washington to consider possible Congressional action relative to pathogenic organisms.

Representive of the Society in Connection with the International Plant Quaran-

tine Committee established under the League of Nations. On recommendation of the Council the Society voted to appoint H. A. Edson as its representative to be available in case the Society should be in a position to assist the important international committee established under the League of Nations, as recommended by the International Botanical Congress at Amsterdam and endorsed by this Society, to consider problems related to plant quarantines and similar regulations.

Representative on the Council of the Third International Congress for Microbiology. On recommendation of the Council the Society voted to appoint B. O. Dodge as its representative on the Council of the Third International Congress to be held in New York City in the summer of 1939.

Eriksson Institute. On recommendation of the Council the Society reaffirmed its previous request that D. Reddick with others selected by him formulate for Society consideration proposals relative to the Eriksson Institute.

Membership Lists. On recommendation of the Council the members present voted without dissent that it be the policy of the Society that its membership list be supplied only to members.

Biological Smoker. On Council recommendation the Society voted a contribution of $10.00 from its treasury for the Biologists' Smoker arranged by the American Society of Naturalists at Indianapolis.

Editorial Policy. On recommendation of the Council the Society passed unanimously the following resolution: The American Phytopathological Society endorses the policy of the Editorial Board and pledges its cooperation in the future continuation of the Board's efforts toward attaining the utmost conciseness consistent with high scientific quality in the papers presented in PHYTOPATHOLOGY.

JOHN JEFFERSON DAVIS

November 4, 1852—February 26, 1937

John Jefferson Davis was graduated from the University of Illinois at its first Commencement in 1872 with the degree of Bachelor of Science. In 1875 he received the degree of Doctor of Medicine from the Hahnemann School of Medicine. From 1877 to 1911 he practiced medicine in Racine, Wisconsin. In 1911 he retired from active practice to accept the Curatorship of the University of Wisconsin Herbarium. Although entitled to retirement, he refused to give up active work and he was at his desk as usual on the day of his death.

His medical training aroused in him a desire to study the flora of Wisconsin and in a short time he was exchanging specimens with many of the better-known botanists of that time. He soon realized that it would be necessary to confine his more intensive studies and he decided upon the Rusts and the Imperfect Fungi. At the time of his death he was internationally recognized as an authority on the latter group.

He very early became affiliated with scientific groups, and for more than 50 years he was in attendance at the national meetings. Dr. Davis was a member of several scientific societies and was a charter member and patron of The American Phytopathological Society. He was president of the Wisconsin State Medical Society and, during the last years of his life, was keenly interested in the studies on human cancer.

Dr. Davis did not publish extensively. His lists of the parasitic fungi of Wisconsin are the best known, and the 20th list was completed just one week before his death. He was at all times an inspiration to the younger mycologists; and, if one is to judge by the numerous letters of appreciation found in his files, his influence was more far-reaching than was suspected, even by his most intimate friends and coworkers. His was a most kindly personality, and all who knew him prized his friendship most highly.

STACY OTTO HAWKINS

September 6, 1899—July 10, 1937

Stacy Otto Hawkins was graduated from Indiana University in 1923 with the degree of Bachelor of Arts and received the degree of Master of Arts from the same institution in 1926. During 1921–22 he taught history and botany at Marion College, Marion, Indiana. From 1925 to the time of his death he was employed by the Florida Agricultural Experiment Station in the Department of Plant Pathol-

ogy, first as Field Assistant and later as Assistant Plant Pathologist. From 1925 to 1936 he served at Homestead, Florida, and early in 1936 he was transferred to Gainesville.

Mr. Hawkins made important contributions toward the control of certain vegetable diseases in Florida, particularly certain diseases of tomatoes. It was truly said of him: "He was an earnest and conscientious worker, and was always ready to encourage a worthy cause and help its advancement in any way he could."

GEORGE PERKINS CLINTON

May 7, 1867—August 13, 1937

George Perkins Clinton was graduated from the University of Illinois in 1890 and received the degree of Master of Science from the same institution in 1894. In 1901 he received the degree of Master of Science and, in 1902, the degree of Doctor of Science from Harvard University.

From 1890 to 1900 Dr. Clinton served as Assistant Botanist in the Illinois Agricultural Experiment Station and Assistant in Botany in the University of Illinois. From July 1, 1902, to June 30, 1937, he served as Botanist of the Connecticut Agricultural Experiment Station. From July 1, 1937, to the time of his death, he was retained on the staff of the same institution as Consulting Botanist. From 1902 to 1925 he was also Botanist for the Connecticut State Board of Agriculture. From 1903 to 1927 he was chairman of the committee on fungous diseases of the Connecticut Pomological Society. From 1919 to the time of his death he was one of the three members of the Connecticut Tree Protection Examining Board. From 1915 to 1926 Dr. Clinton was Lecturer in Forest Pathology and, from 1926 to 1929, Research Associate in Botany in Yale University. Dr. Clinton also served temporarily, both in the United States and in foreign countries, on special assignments for the U. S. Department of Agriculture and for Harvard University.

In addition to being a sustaining life member of The American Phytopathological Society, Dr. Clinton was an honored member in numerous other scientific and agricultural societies. It was well said of him that he was "sincere, honest, generous, loyal, democratic, determined, and just." He also had a keen sense of humor and an intense hatred of pretense. He was deeply religious and for those in sorrow, he was tenderly sympathetic.

FRITZ SATTLER

June 3, 1905—September 12, 1937

Fritz Sattler studied natural sciences in the Universities of Tübingen and Freiburg (Germany) from 1925 to 1931. He went to Bonn in 1932 where he studied plant pathology at the Institut für Pflanzenkrankheiten. In 1934 he went to Giessen where he completed his graduate study and received his doctorate in 1935.

From 1935 to 1936 he served in the Abteilung für Pflanzenkrankheiten des Institutes für Pflanzenbau und Pflanzenzüchtung der Ludwigs-Universität, Giessen. In 1937 he was employed as plant pathologist and agronomist by the seed firm of Gebr. Dippe A. G. in Quedlinburg, Germany.

Dr. Sattler was a man of sterling qualities, untiring diligence, and, withal, showed great promise. His untimely death cut him off in the vigor of life.

JACOB JOSEPH TAUBENHAUS

October 20, 1885—December 13, 1937

Jacob Joseph Taubenhaus was graduated from the National Farm School, Doylestown, Pennsylvania, in 1904. He received the degree of Bachelor of Science in 1908 and the degree of Master of Science in 1909, both from Cornell University. He was granted the degree of Doctor of Philosophy by the University of Pennsylvania in 1913.

From 1913 to 1916 he was, at first, Assistant Pathologist, and, later, Associate Pathologist at the Delaware Agricultural Experiment Station. In 1916 he was Russell lecturer for the Massachusetts Horticultural Society. From 1916 to the time of his death, he was Chief of the Division of Plant Pathology and Physiology at the Texas Agricultural Experiment Station. From 1924 to 1937 he was also a member of the graduate faculty of the Texas Agricultural and Mechanical College.

Dr. Taubenhaus was an unusually enthusiastic worker. He did notable work on the diseases of the sweet pea, sweet potato, and cotton. In addition to being a charter member of The American Phytopathological Society, he was a member of a number of other scientific and agricultural societies. He was deeply religious and active in Zionist affairs.

ERRATA, VOLUME 27

Page 15, table 6, footnote a, *read* 50[1]—From same host etc. *for* 50—From same host etc.

Page 112, line 10 *read* His spring *for* This spring.

Page 148, table 1, *read*

Group and species	Moderately resistant	Susceptible
Chickasaw plum, *Prunus angustifolia* Marsh	×	
Sumac, *Rhus* sp.		×
for		
Chickasaw plum, *Prunus angustifolia* Marsh		×
Sumac, *Rhus* sp.		

Page 584, line 6, *read* cupric *for* cuprous.

Page 735, line 16, *read* Lind[5] *for* Lind[2].

Page 1003, table 1,

read	Himalaya	Mild	Extreme
for	Himalaya	"	Extreme

Page 1016, line 6, also page 1020, table 4, *read* Pyronema *for* Pyronena.

Page 1120, table 1, *read* Cerelose *for* Cerulose.

Page II of Index, column 1, 4th line from bottom, *transfer* tabacum (See also Wildfire) to 13th line from bottom, to *read* stewarti, etc.
tabacum (See also Wildfire.)

Page IV,[1] column 1, line 24 *read* Chestnut, breeding *for* breeding.

Page XIII,[1] column 1, 5th line from bottom *read* 109 *for* 948.

Page XX,[1] column 1, line 42, *read* Virulence, factors *for* factors.

Page XXI,[1] column 2, *read* 729 *for* 739.

THE MODE OF ACTION OF BORDEAUX ON MYCOSPHAERELLA FRAGARIAE

A. G. PLAKIDAS

(Accepted for publication February 1, 1938)

INTRODUCTION

Earlier studies at the Louisiana Agricultural Experiment Station (13, 14) have shown (1) that infection with the strawberry leaf-spot fungus, *Mycosphaerella fragariae* (Tul.) Lindau, takes place primarily, if not wholly, through the dorsal surface of the leaf, and (2) that the disease can be controlled effectively by spraying with Bordeaux mixture, even though, with the system of spraying practiced by the strawberry growers in Louisiana, very little of the spray material actually reaches the dorsal side of the leaves. These two observed facts appear contradictory, since the action of Bordeaux spray is generally assumed to be protective, and yet, in this case control was obtained without protecting the surface of the leaf through which infection occurs.

In the hope of obtaining information that might explain the apparent inconsistency in the results of previous studies, an investigation was undertaken to determine the manner in which Bordeaux spray acts on the conidia of *Mycosphaerella fragariae*. Under Louisiana conditions where the strawberry plants remain active during the winter, the dissemination and perpetuation of the fungus depend almost solely on conidia. The primary object of this investigation was to determine the action of copper compounds, particularly that of Bordeaux mixture, on the conidia. It was felt that a knowledge of the tolerance of these spores for the fungicide would be a contribution to a better understanding of the way in which control is obtained by spraying and might lead to a more efficient method of controlling this important disease. The methods and results of this investigation form the substance of this paper. A brief abstract of the subject has been published (15).

MATERIALS AND METHODS

Source of Spores. The spores used in the various tests were obtained from naturally infected leaves collected in the field. The leaves were washed under running water with a brush and placed in moist chambers. The washing, besides removing the grit and other foreign material and leaving the surface clean, served the additional purpose of removing the old spores so that those spores that were produced in the moist chambers were of approximately the same age when used in the toxicity tests. In this way variability due to age of spores was largely eliminated. In about 24 hours after the leaves were placed in the moist chamber, spores were produced in great profusion. These were picked by means of a closely cropped camel-hair brush and made into the desired suspension. It was found that the most uniform germination was obtained with spores 24 to 36 hours old; spores of this age

were used in most of the tests. The density of the suspension was obtained by making microscopic mounts and examining with an intermediate objective (Zeiss No. 20). Optimum germination was obtained when approximately 10 spores to the field were present.

Water. The spores germinated well (usually over 90 per cent) in tap water and, so, in the tests with Bordeaux mixture, tap water was used for the checks and also for the preparation of the Bordeaux mixture and the lime suspension. However, since the Baton Rouge water is rather strongly alkaline (pH 8.0–8.5), tap water could not be used in the tests involving high dilutions of soluble copper compounds, such as copper sulphate and copper chloride, because precipitation of the copper occurred. Germination in distilled water was erratic. Usually the spores germinated as well in distilled as in tap water; but, occasionally, poor or irregular germination occurred in distilled water. For this reason, rain water (when available) or tap water, rendered slightly acid (pH 6.5) by the addition of hydrochloric acid, was used in the series of tests with high dilutions of soluble copper compounds. The spores germinated well both in rain water and in acidified tap water.

Glassware. The importance of clean glassware in spore germination tests has been well emphasized by McCallan (8) and other workers.

The slides and other glassware were cleaned by boiling in dichromate cleaning mixture then washing successively in several changes of tap water, alkaline water, distilled water, and 95 per cent alcohol. With the slide perfectly clean, the drop of spore suspension placed on it would spread out evenly, forming a thin film that provided uniform aeration conditions; with a slide not thoroughly clean, the suspension would form a round, heavy drop that did not allow uniform aeration.

The slides were placed on glass-rod racks in large (21 cm. diameter) moist chambers, and were examined for germination usually at the end of 24 hours, and in certain cases reexamined at the end of 36 or 48 hours. In the beginning, 8 slides were used for each series. However, since the variation in the percentage of germination between the different slides of a series was found to be very small, the number of slides per series was reduced to 4 and the tests were repeated. The germination counts were made by placing each slide on the mechanical stage of the microscope, counting all the spores that appeared on one microscopic field, then so moving the slide that the same field was not counted twice. Usually 100 spores were counted per slide, so that the average germination was obtained from a count of 400 or 800 spores, depending on whether 4 or 8 slides were used per series.

EXPERIMENTAL

Exploratory tests were first carried out for the purpose of obtaining some leads as to the manner in which Bordeaux acts on *Mycosphaerella fragariae*, for, as already stated, effective control of leaf spot is obtained by spraying with Bordeaux mixture, even though the spray does not cover the under sur-

face of the leaves through which the infection takes place. These preliminary tests were intended primarily to answer the following questions: (1) Does the toxic principle of the spray penetrate the leaf tissue to kill the fungus in it? (2) Are spores produced on the sprayed leaves? (3) If spores are produced on the sprayed leaves, are they viable, *i.e.,* will they germinate if placed in a favorable medium?

EFFECT OF BORDEAUX ON INTERNAL MYCELIUM

The first question was easily answered. Spotted leaves that had been sprayed twice, January 2 and 10, were collected in the field on January 13, brought to the laboratory, and washed thoroughly in running water with a brush to remove the spray residue. Then, pieces of tissue from the younger spots were cut, surface-disinfected in 1–1,000 mercuric chloride for ½ minute, washed with sterile water, and placed on bean-pod agar. The fungus was easily isolated, which made it apparent that the mycelium within the tissue was not killed by the spray.

COMPARISON OF THE NUMBER OF SPORES ON SPRAYED AND NON-SPRAYED LEAVES

The following procedure was followed in making comparative counts of the number of spores on sprayed and nonsprayed leaves: A definite number of spots (usually 5) of as nearly as possible the same size and age were selected from each lot, and their upper surfaces were brushed with a close-cropped camel-hair brush into a watch glass containing 0.5 cc. of water. One drop (1 20 cc.) of the spore suspension was placed on a slide and covered with a 22 mm. cover slip. This amount of spore suspension was just enough to form an even film between the slide and the cover slip. The slide was then placed on the microscope, which was equipped with a mechanical stage, and examined with a Zeiss No. 20 objective, and the spores appearing in a field counted. The slide was then moved to other fields, care being taken not to count the same field twice.

While it is realized that this method is empirical and relatively crude, it is considered satisfactory for purposes of comparison, since the same procedure was followed with both the sprayed and the nonsprayed leaves, and since the many times repeated tests gave consistent results. The following example illustrates the reliability of this method. When 5 spots were brushed to make the spore suspension, the average number of spores per microscopic field (average of 40 fields) was 2.55 and 0.27, respectively, for the nonsprayed and the sprayed leaves; when 10 instead of 5 spots were used from the same lots of leaves, the average number of spores per microscopic field (average of 50 fields) was 4.60 and 0.62, respectively. These figures are roughly double those obtained when 5 spots were used.

From the results of many different tests, it was concluded that relatively few spores are produced on the upper surface of a sprayed leaf. Between 7 and 10 times as many spores were found on the nonsprayed as on the sprayed leaves. It is believed that when moisture is present on the sprayed

leaves, spray hinders normal growth of the sporogenous hyphae, thereby inhibiting sporulation to a great extent and reducing considerably the amount of potential inoculum at its source. In this way, Bordeaux may be considered to act more as an eradicative than as a protective fungicide. Table 1 shows the results obtained from a series of tests that may be considered as representative of many others. The material for this series was

TABLE 1.—*Comparison of the number of spores of Mycosphaerella fragariae on sprayed and nonsprayed strawberry leaves*

Microscopic field	Sprayed				Nonsprayed			
	Test I	Test II (Same suspension as in I, but different slide)	Test III (New suspension)	Test IV (Same suspension as in III, but different slide)	Test I	Test II (Same suspension as in I, but different slide)	Test III (New suspension)	Test IV (Same suspension as in III, but different slide)
1	1	3	1	2	6	10	4	7
2	1	0	1	0	6	3	12	3
3	2	0	0	0	2	6	5	8
4	0	1	0	1	6	4	9	9
5	2	2	4	2	3	8	6	5
6	0	0	0	0	11	2	3	2
7	0	3	2	0	9	5	10	4
8	0	0	1	0	2	9	5	6
9	0	0	0	2	5	6	7	10
10	0	1	0	0	7	7	5	3
	Average for the 4 tests (40 fields) 0.8				Average for the 4 tests (40 fields) 6.00			

collected in the field at Hammond, Louisiana, on January 19, 1935, and consisted of leaves of nonsprayed plants and from plants that had been sprayed twice (January 2 and 10) with a 4–4–50 Bordeaux mixture. The sprayed and nonsprayed plants were of the same variety (Klondike) and were growing on adjoining rows under identical conditions.

VIABILITY OF SPORES PRODUCED ON SPRAYED LEAVES

When sprayed, spotted leaves were placed in a moist chamber with the humidity high enough to stimulate growth, but not so high as to form a film of moisture on the sprayed surfaces, spores were produced in abundance on both the upper and the lower surfaces, those on the upper (sprayed) surface pushing out through the dry spray residue. When such spores were picked at the point of a needle, care being taken not to include any spray particles, and placed in water on slides, they germinated well, showing that they were viable. If, however, the sprayed, spotted leaves in the moist chamber were sprayed with water by means of an atomizer so as to wet the spray residue and form a thin film of moisture on their surfaces, no spores were produced, except on elevated areas where the film of moisture evaporated, leaving the spray residue dry. Where the leaf was level, so that the film of moisture

covered the surface and kept the spray residue wet, no spores were produced either on the upper or under surface. Since no spray material was present on the under leaf surface, it was apparent that some of the copper in solution must have diffused through the dead tissue of the spots and reached the under surface. Furthermore, if the upper surfaces of the sprayed, spotted leaves in the moist chambers were atomized with water after spores had been produced, and allowed to stay for sometime (usually overnight) it was found that the spores on the upper surface were invariably killed, for they never germinated when transferred to water on slides.

The germination of the spores on the under leaf surface was variable. When slides were prepared from separate spots, the percentage of germination varied greatly, from no germination at all, in some cases, to nearly as high as that of the checks in others. This would indicate that the copper in solution does not become equally distributed on the under leaf surface.

That the data obtained from the laboratory tests give a true picture of what happens under field conditions, was brought out by the following experiment. On February 7, 1934, leaves were collected from sprayed (4 times) and nonsprayed plants in the garden in Baton Rouge, brought to the laboratory, and slides for germination tests were prepared immediately. Heavy dew had fallen during the night, and, when the leaves were collected at 8:00 a.m., a thick film of moisture occurred on their upper, and droplets of moisture on their under surfaces. Spores were picked from the under leaf surface only and placed in tap water on slides. Six series of slides (4 slides to each series) were thus prepared from as many separate leaves. Table 2 shows the results obtained.

TABLE 2.—*Comparison of the germination of spores of Mycosphaerella fragariae produced on the under surfaces of Bordeaux-sprayed and nonsprayed leaves under field conditions*

Series	Percentage germination (ave. of 4 slides)	
	Sprayed	Nonsprayed
1	4.2	92.1
2	1.7	88.7
3	61.7	95.4
4	7.2	93.8
5	72.6	96.0
6	9.3	91.8

These results are similar to those obtained with spores produced on leaves in moist chambers in the laboratory and indicate that, even though no spray residue may be visible on the under surfaces of the sprayed leaves, some of the soluble copper must reach and kill at least some of the spores formed on the under surface. That the spores that failed to germinate were already dead, rather than that germination was inhibited by the chance inclusion of some spray residue particles in the spore suspension on the slides, seemed evident from the fact that the germ tubes of the spores that did germinate

developed normally, even in the series where the percentage of germination was very low. For example, in series 2, where the percentage of germination was the lowest (1.7 per cent) two spores had, at the end of 34 hours, long, branched germ tubes, the longest branches of which were 274 μ and 484 μ, respectively.

TESTS WITH BORDEAUX MIXTURE

After the preliminary tests had indicated that *Mycosphaerella* spores are sensitive to copper toxicity, a long series of experiments extending from 1934 to 1937 were carried out in order to determine more accurately the action of Bordeaux mixture on the spores. Various forms of Bordeaux, namely, freshly prepared, aged on glass slides for different periods, freshly sprayed on leaves, and weathered on leaves was used in these experiments.

DIRECT CONTACT WITH THE BORDEAUX PARTICLES

Germination was completely inhibited when the spores came in direct contact with Bordeaux mixture, irrespective of whether it was freshly-prepared, aged on slides, or weathered on leaves. With freshly-prepared mixture, germination was inhibited at as high a dilution as 1 part of a 4–4–50 suspension in 1000 parts of water. The results of a few experiments, which are typical of many performed, are presented.

Experiment I. Tests with Fresh 4–4–50 Bordeaux

A. Check: Spores in tap water. Ave. germination 92.7%; ave. length of germ tubes 157.3 μ.

B. Spores in undiluted and in diluted solutions of 1–100, 1–200, 1–500, and 1–1000 4–4–50 Bordeaux. No germination.

C. Spores in 1–10,000 dilution of 4–4–50 Bordeaux. Ave. germination 84.3%; ave. length of germ tubes 54.7 μ.

D. Spores in 1–20,000 dilution of 4–4–50 Bordeaux. Ave. germination 87.0%; ave. length of germ tubes 86.6 μ.

E. Spores in 1–40,000 dilution. Ave. germination 92.7%; ave. length of germ tubes 159.8 μ.

F. Spores in 1–80,000 dilution. Ave. germination 93.0%; ave. length of germ tubes 161.3 μ.

G. Spores in 1–100,000 dilution. Ave. germination 92.3%; ave. length of germ tubes 153.0 μ.

These results illustrate the extreme sensitivity of the spores of *Mycosphaerella fragariae* to copper toxicity. No germination occurred when the spores were in contact with fresh Bordeaux, even at the high dilution of one part of 4–4–50 Bordeaux suspension in 1000 parts of water; and, although a high percentage of the spores germinated at the 1–10,000 and 1–20,000 dilutions, some degree of toxicity was apparent, even at these extremely high dilutions, as judged by the length of the germ tubes. At the 1–20,000 dilution the average length of the germ tubes was approximately one-half that of the check.

It is admitted, of course, that the concentration of Bordeaux in dilutions is only approximate. It is difficult to dilute coarse suspensions, such as Bor-

deaux mixture, which have variable-size particles, and get the exact concentration of the suspension in the desired dilution. Notwithstanding this difficulty, it is apparent that extremely small amounts of the Bordeaux suspension were present in dilutions of 1–10,000 and 1–20,000; yet this was sufficient to manifest a certain degree of toxicity. No toxic action was apparent in dilutions of 1–40,000 or higher.

Experiment II. Tests with Aged Bordeaux. Measured amounts (1/20 or 0.1 of a cc.) of a 4–4–50 Bordeaux were smeared on glass slides and allowed to dry. The slides were then stored in a slide box for subsequent use. Table 3 shows the plan and the results obtained from a typical test with aged Bordeaux.

TABLE 3.—*Effect of lime and of dried, aged 4–4–50 Bordeaux on the germination of conidia of Mycosphaerella fragariae*

Treatment	Percentage germination (ave of 4 replications)
A. Check—spores in distilled water	95.2 ± 2.12
B. " " " tap "	89.2 ± 5.29
C. Spore suspension on 1/20 cc. 1% lime smear, dried and aged for 5 days. Stirred with a camel-hair brush to mix the spores with the lime particles	94.5 ± 2.07
D. As in "C," but lime smear not stirred	96.0 ± 1.7
E. Spore suspension on 0.1 cc. lime smear; stirred	94.6 ± 1.85
F. " " " " " " " not stirred	95.7 ± 1.48
G. " " " " " Bordeaux smear, dried and aged 5 days; stirred	0
H. As in "G," but not stirred	0
I. Spores on 1/20 cc. Bordeaux smear, aged 5 days; stirred	0
J. " " " " " " " " " not stirred	0
K. " " " " " " aged one year; stirred	0
L. " " " " " " " " " not "	0
M. " " 0.1 " " " " " " stirred	0
N. " " " " " " " " " not "	0

Germination was completely inhibited when a water suspension of spores was placed on dried, aged-Bordeaux smears on glass slides. The results were the same, regardless of the amount of Bordeaux smeared on each slide (1/20 or 0.1 of a cc.) and the period of aging of the smears (5 days or 1 year), and regardless of whether the smear was stirred, so as to bring the spray particles into intimate contact with the spores, or left unstirred.

The lime smears (Table 3, C–F) were included in these tests in order to determine if the lime component of Bordeaux mixture exerted any toxic action against the spores. Several workers have found lime in certain concentrations to be toxic to fungi and bacteria. Lutman (7) noted lime toxicity to spores of *Phytophthora omnivora* and *Sclerotinia cinerea*. McCallan (9) found that C.P. calcium hydroxide in concentrations higher than 0.1 gm. per liter and C.P. calcium sulphate in concentrations higher than 0.7 gm. per liter were toxic to spores of *Sclerotinia americana*; and Keitt, Pinckard, and Riker (6) have shown that a 5-minute exposure of *Erwinia amylovora* to

M/2.16 calcium hydroxide was lethal. As shown in table 3, lime was not toxic to *Mycosphaerella* spores. The percentages of germination in the lime series were as high as those of the water checks, and the germ tubes were long and normal.

Both freshly prepared 4–4–50 Bordeaux and freshly slaked 1 per cent lime were found to have the same reaction, pH 12.1 (determinations made by means of the Coleman glass electrode). After aging (sprayed on glass, allowed to dry, and age for 5 days in the laboratory, then suspended in a volume of water equal to the original) the pH of both 4–4–50 Bordeaux and 1 per cent lime dropped to 9.5. This relatively high alkalinity evidently had no adverse influence on the germination of the spores.

Experiment III. Tests with Weathered Bordeaux. Goldsworthy and Green (5) have noted that the toxicity of Bordeaux mixture to conidia of *Sclerotinia fructicola* diminishes with the exposure of the spray residue to field weathering and rainfall. In order to determine the effect of weathered Bordeaux on the spores of *Mycosphaerella fragariae* sprayed strawberry leaves were collected on December 18, 1936, dried, and stored in paper bags in the laboratory. The last spraying of the plants from which the leaves were collected was done on October 29, 1936, so the spray had been exposed to weathering in the field for 50 days. One part of the field was sprayed with 4–4–50 Bordeaux alone and the other with the same mixture to which an adhesive clay ("Wyo-Jel") was added at the rate of 10 pounds per 100 gallons of spray. On the date of collecting, the spray residue was barely visible on the leaves sprayed with Bordeaux alone, while considerable residue remained on those sprayed with the Bordeaux–"Wyo-Jel" mixture.

On February 5, 1937, these tests were made using the dried, stored leaves:

(1) Spray residue was brushed off the dried leaves and suspensions were prepared using 0.1 g. of the residues in 2 cc. of distilled water. Spores were placed on slides in these suspensions.

(2) Portions of the dried leaves were crumpled into Syracuse dishes in 6 cc. of water, let steep for 36 hours, then filtered through filter paper. Spore suspensions were made in the filtrates and placed on slides in moist chambers.

The results of the tests with weathered Bordeaux are shown in table 4. Germination was completely inhibited by Bordeaux spray residue that had weathered on leaves outdoors for 50 days and aged in the laboratory for an additional period of 49 days. The residue had been exposed to 4.69 inches of rain—it rained 4 times (total 2.07 inches) in November and 5 times (total 2.62 inches) in December. That the spores germinated relatively well in the filtrate of the leaves sprayed with the mixture containing the "Wyo-Jel" clay, while they did not germinate at all in the filtrate of the leaves sprayed with Bordeaux alone was probably due to the fact that the clay acted as a filter, adsorbing and taking out of solution the small amount of soluble copper present. The filtrate of the leaves sprayed with Bordeaux alone was dark brown, while that of the leaves sprayed with the Bordeaux–"Wyo-Jel" mixture was nearly colorless, indicating that even the color of the solution was adsorbed by the "Wyo-Jel" clay.

TABLE 4.—*Effect of weathered Bordeaux mixture residue on the germination of conidia of Mycosphaerella fragariae*

Treatment	Percentage germination (ave. of 4 replications)
A. Check—spores in tap water	93.2 ± 1.8
B. Check—spores in distilled water	91.5 ± 2.7
C. Spores in weathered Bordeaux residue	0
D. Spores in weathered Bordeaux plus "Wyo-Jel" residue	0
E. Spores in filtrate of nonsprayed leaves	94.1 ± 2.06
F. Spores in filtrate of leaves sprayed with Bordeaux alone	0
G. Spores in filtrate of leaves sprayed with Bordeaux plus "Wyo-Jel"	83.3 ± 6.3[a]

[a] The percentage of germinated spores differed considerably between the 4 slides of this series, as seen by the large standard deviation, and the germ tubes were shorter than those of the checks; otherwise, the germination was normal.

Experiment IV. Spore Germination on Leaves. All the germination tests reported above were made on glass slides, *i.e.*, under unnatural conditions. In order to simulate field conditions as nearly as possible the following experiment was carried out. Strawberry leaves were collected in the field on March 4, 1937, and divided into 3 lots, *viz.*:

Lot 1. Nonsprayed leaves.

Lot 2. Leaves sprayed with 4–4–50 Bordeaux on February 22, *i.e.*, after 10 days of weathering.

Lot 3. Leaves sprayed last on March 4, collected about 2 hours after spraying, just as soon as the spray had dried thoroughly.

The leaves were placed in Petri dishes in moist chambers, care being taken not to disturb the spray residue film, and a few drops of a suspension of spores of *Mycosphaerella fragariae* in tap, rain, or distilled water were placed on their surfaces. After 24 hours, representative samples of the spore suspensions were transferred by means of a pipette from the leaves onto glass slides and examined for germination (Table 5). No germination occurred on the

TABLE 5.—*Germination of conidia of Mycosphaerella fragariae on the surface of Bordeaux-sprayed and nonsprayed strawberry leaves*

Treatment	Percentage germination (ave. of 6 replications)
A. Check—spores in distilled water on nonsprayed leaves	72.6
B. Check—spores in tap water on nonsprayed leaves	75.2
C. Check—spores in rain water on nonsprayed leaves	88.0
D. Spores in distilled water on leaves sprayed Feb. 22 (10 days' weathering)	0
E. Spores in tap water on leaves sprayed Feb. 22 (10 days' weathering)	0
F. Spores in rain water on leaves sprayed Feb. 22 (10 days' weathering)	0
G. Spores in distilled water on leaves sprayed March 4 (no weathering)	0
H. Spores in tap water on leaves sprayed March 4 (no weathering)	0
I. Spores in rain water on leaves sprayed March 4 (no weathering)	0

sprayed leaves. The spores appeared dead, with their contents plasmolyzed and coagulated. The percentage of germination in the checks (nonsprayed leaves) was lower than that usually occurring on glass slides, but this is believed to be due to the fact that spores with long germ tubes would tend to adhere to the leaf surface, and, in transferring the spore suspension from the leaf to the glass slide, a larger proportion of nongerminated spores would be picked by the pipette.

In order to determine if the dead-appearing nongerminated spores (Series D–I, Table 5) were actually killed or if germination was merely inhibited, the suspensions from several leaves from series D, E, and F and from series G–I, respectively, were combined on filter paper in a funnel and the spores were washed for ½ hour with running water. The spores were then placed on slides and examined 24 hours later to see if they would germinate. Checks were provided (1) by placing spores in the last washings of the Bordeaux-exposed spores and (2) by washing fresh spores in water on filter paper for the same length of time as the Bordeaux-exposed spores. Good germination was obtained in both checks. The Bordeaux-exposed, washed spores did not germinate. This indicates that they were actually killed rather than that germination was inhibited by a 24-hour exposure to Bordeaux residue on leaves.

ACTION OF BORDEAUX AT A DISTANCE

Under this heading are discussed, for the sake of convenience, miscellaneous experiments in which the spores were not brought in direct contact with the spray particles, but were either separated from them by a water bridge or by 1 or 2 thicknesses of paper, or were exposed to the supernatant, clear liquid of fresh or dried Bordeaux.

TOXIC ACTION OF BORDEAUX THROUGH WATER BRIDGE

That Bordeaux can exert its toxic action at a distance from its solid particles through a film of water has been shown by Aderhold (1), Lutman (7), McCallan (9), and others. Barker and Gimingham (3), on the other hand, have maintained that actual contact, or at least very close association, is necessary for toxic action. McCallan's (9) method was used to determine if Bordeaux was toxic to conidia of *Mycosphaerella fragariae* at a distance.

One drop (approx. 0.05 cc.) of a 4–4–50 Bordeaux mixture was placed on each of several slides and allowed to dry for a week. A spore suspension in distilled water was prepared and a drop of it was placed on the same slides at distances of 1, 2, 5, and 10 mm. from the Bordeaux spot. After waiting for about 10 minutes to allow the spores to settle, a drop of tap water was placed on the Bordeaux spot, and a narrow bridge of water (approx. 2 mm. in width) was made by means of a fine pipette connecting the drop of spore suspension with the drop of water on the Bordeaux spot. In one series, 2 drops of spore suspension were placed on the slides, the first drop 3 mm. from the Bordeaux spot and the second 3 mm. from the first. The first spore-

suspension drop was connected to the Bordeaux spot on one side and to the second spore drop on the other by means of a narrow bridge or film of water, as shown in figure 1. The drops were approximately 10 mm. in diameter.

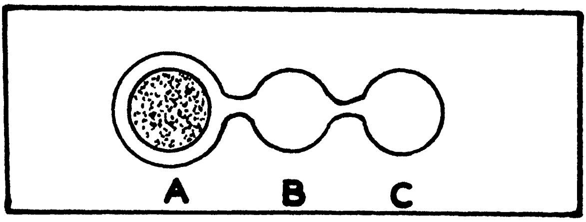

FIG. 1. Diagram showing the arrangement of the Bordeaux smear and the two drops of spore suspension on the slide. A. Bordeaux smear and film of water over it. B. First spore drop located 3 mm. from the Bordeaux smear and from the second spore drop (C) and connected with them by narrow water film bridges. The diameter of the spore drops was approximately 10 mm., so the distance between the Bordeaux smear and the second spore drop was approximately 16 mm.

Therefore, the second spore drop was approximately 16 mm. from the Bordeaux spot. In making the water bridges, care was taken to move the pipette from the spore suspension drop toward the Bordeaux spot so as to prevent any loose Bordeaux particles from floating toward the spore drops. The slides were placed in moist chambers and examined 24 hours later.

In connection with this experiment, spore suspensions were made in the supernatant clear liquid of freshly prepared 4–4–50 Bordeaux, and also in the supernatant liquid of dried 4–4–50 Bordeaux. The latter was obtained by placing small amounts of a 4–4–50 Bordeaux mixture in Syracuse watch glasses, so rotated that the sides and bottoms became well coated, and allowed to dry and age for a week. Distilled water was placed in the Bordeaux

TABLE 6.—*Germination of conidia of Mycosphaerella fragariae in water connected by means of a water film bridge with a spot of dried Bordeaux and in the supernatant liquids of fresh and dried 4–4–50 Bordeaux*

Treatment	Results
A. Check—spores in tap water	Ave. germination 94.6 ± 1.8
B. Check—spores in distilled water	" " 87.7 ± 2.3
C. Spore drop 1 mm. from Bordeaux	No germination; spores unchanged.
D. Spore drop 2 mm. from Bordeaux	No germination; spores unchanged.
E. Spore drop 5 mm. from Bordeaux	No normal germination; spores swollen, some with "buds"[a] (Fig. 2, D)
F. Spore drop 10 mm. from Bordeaux	No normal germination; spores swollen, some with short germ tubes (Fig. 2, E)
G. Two separate spore drops connected to the Bordeaux spot in series as shown in figure 1	No germination in first drop. In the second drop germination was approx. same as in series "F."
H. Spores in supernatant liquid of fresh Bordeaux	No germination.
I. Spores in supernatant liquid of dried Bordeaux	No normal germination; spores swollen, some with short, curled, twisted germ tubes (Fig. 2, C)

[a] The term "bud" is used in the sense used by Goldsworthy and Green (5) to indicate the primordial germ tube or the beginning of germination.

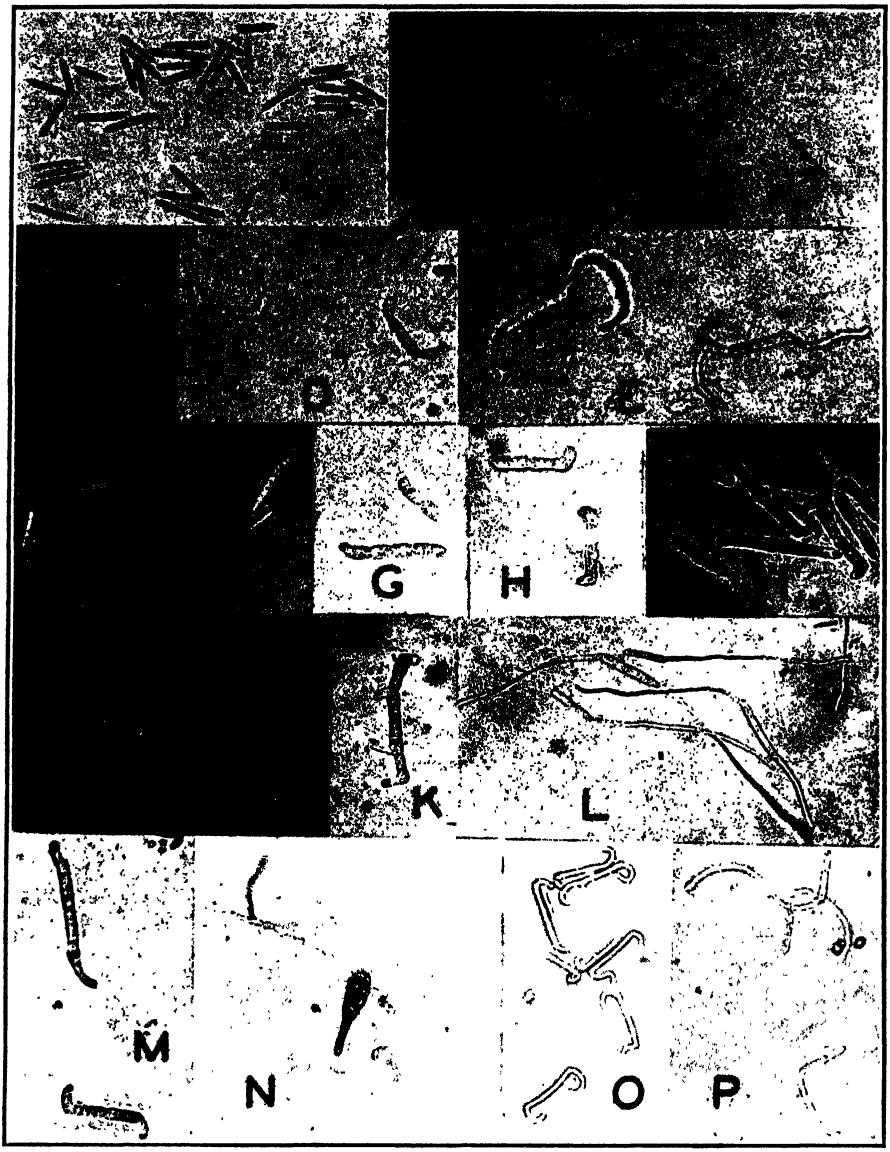

FIG. 2. Copper toxicity to conidia of *Mycosphaerella fragariae*. A. Normal ungerminated 24-hour-old conidia. × 300. B. Normal germination in tap water in 24 hours, showing long and relatively straight germ tubes. × 330. C. Germination in the supernatant liquid of dried Bordeaux in 24 hours. Note the extreme swelling of the conidia and the peculiar curling of the germ tubes. × 330. D. Germination at a distance of 5 mm. from the Bordeaux smear in 24 hours. The growth of the germ tubes did not go beyond the "bud" stage. × 330. E. Germination at a distance of 10 mm. from the Bordeaux smear in 24 hours. Short germ tubes were formed, but no further growth occurred. × 300. F. Conidia in 4 p.p.m. of $CuSO_4$. No germination and no swelling in 24 hours. × 330. G. Swelling but no germination of conidia in 3.33 p.p.m. of $CuSO_4$ in 24 hours. × 330. H. Swelling and "budding" but no normal germination in 2 p.p.m of $CuSO_4$ in 24 hours. × 330. I. Conidia in 1 p.p.m of $CuSO_4$. Swelling and "budding" but no normal germination in 24 hours. × 380. J. Abnormal germination of conidia in 0.5 p.p.m. of $CuSO_4$ in 24 hours. Note the curling and twisting of

coated dishes and allowed to stand for 48 hours, then some of the clear supernatant liquid was drawn off by means of a pipette and used to make spore suspensions. The results of these tests are summarized in table 6. No germination and no swelling of the spores occurred in the supernatant liquid of fresh Bordeaux, and when the spore-suspension drop was 1 or 2 mm. from the Bordeaux spot. At 5-mm. distance, the spores swelled considerably and some began to germinate, but growth stopped at the "bud" (primordial germ tube) stage (Fig. 2, D). At 10-mm. distance, approximately 40 per cent of the spores germinated, but the growth of the germ tubes soon stopped; the germ tubes were no longer at the end of 30 hours than at the end of 24 hours. Germination was not normal in the supernatant liquid of dried Bordeaux (Table 6, I). The spores swelled greatly, and those that germinated had thickened, curled, twisted germ tubes (Fig. 2, C). This peculiar type of germination has been observed in all cases in which the concentration of copper in the solution was on the border line—not enough to inhibit germination but enough to prevent normal growth of germ tubes. A spore germinating normally in water swells somewhat but not greatly, a germ tube being pushed out from one end, and a little later a second one from the other end. The 2 tubes grow out relatively straight (Fig. 2, B) without curling or twisting, and usually no branching occurs during the first 24 to 30 hours. In copper solutions several characteristic abnormalities have been noted. In relatively high concentrations (1–200,000 copper sulphate, for example) the spores are killed and no germination occurs. In higher dilutions (1–200,000 to 1–1,000,000 copper sulphate, for example) the spores swell and enlarge greatly, and "buds" of germ tubes appear, but no further growth takes place (Fig. 2, G–I). Often germ-tube "buds" develop not only from the ends of the spores but also from their sides (Fig. 2, C, K, N, O). Germination from the sides of the spore has never been observed in the checks. In still higher dilutions (those approaching the upper limits of toxicity, 1–2,000,000 copper sulphate, for example) some germination occurs, but growth of the germ tubes soon stops, and the germ tubes curl in a very characteristic manner (Fig. 2, J, O). The reason for this peculiar curling of the germ tubes has not been ascertained.

On the slides containing 2 drops of spore suspension connected in series to the Bordeaux spot (Fig. 1), no germination occurred in the first drop (3 mm. from the Bordeaux spot); in the second drop (16 mm. from the Bordeaux spot) the spores were swollen and about 50 per cent had germinated with short germ tubes. It is evident that where the spores germinated at a distance (5, 10, and 16 mm., Table 6, E–G) germination occurred before suffi-

the short germ tubes. ×330. K. Germination in 0.67 p.p.m. of $CuCl_2$ in 24 hours. Note the formation of germ-tube buds both from the ends and the sides of the conidium. ×330. L. Normal germination of conidia in 0.25 p.p.m. of $CuSO_4$. ×300. M. Germination in 0.5 p.p.m. of $Cu(CH_3COO)_2 \cdot H_2O$ in 24 hours. ×380. N. Germination in 0.5 p.p.m in $Cu(CH_3COO)_2 \cdot H_2O$ in 24 hours. The *Alternaria* sp. spore germinated well in this dilution, while the *Mycosphaerella fragariae* spore produced only short "buds." ×330. O and P. Germination in 0.33 p.p.m. of $Cu(CH_3COO)_2 \cdot H_2O$ after 24 and 30 hours, respectively. ×330.

cient copper ions had diffused through the water bridge to inhibit further growth of the germ tubes; for, the greater the distance between the spore suspension and the Bordeaux spot the longer were the germ tubes.

At the end of 24 hours, half of the slides in each of series F, G, and I, table 6, were treated with a drop of 1–1,000 mercuric chloride to stop further growth of the germ tubes and the other half were left untreated for comparison, and all were reexamined at the end of another 24-hour period. No elongation of the germ tubes occurred in series F and G (10 mm. and 16 mm., respectively, from the Bordeaux spot). Some further growth of the germ tubes occurred in series I (supernatant liquid of dry Bordeaux), though it was not normal, the germ tubes being much shorter than those of the checks and considerably curled and twisted. No attempt was made to determine if the higher toxicity exhibited by the distilled water in contact with the dry Bordeaux (Series F and G, Table 6) relative to the supernatant liquid of dry Bordeaux (Series I, Table 6) was due to any solubilizing action of the conidia on the Bordeaux, as shown by McCallan (9) and McCallan and Wilcoxon (10) for the spores of other fungi, or to possible cumulative action of the copper. McCallan (9) attributed the toxic action of filtrate from dried Bordeaux to conidia of *Sclerotinia americana* to soluble calcium salts or to the alkalinity of the filtrate. In the studies here reported the toxicity of the dry Bordeaux filtrate cannot be attributed to soluble calcium or to alkalinity, because it was found that *Mycosphaerella* conidia germinated well in a suspension of lime (Table 3). Tests with chromotropic acid (2) showed the presence of copper in the supernatant liquid of dry Bordeaux, although the amount was not definitely determined. McCallan and Wilcoxon (10) found 0.2 to 0.3 p.p.m. of copper in distilled water that had remained in contact with dry Bordeaux in glass chambers overnight. In these tests, the amount of soluble copper in the supernatant liquid of dry Bordeaux was probably less than that found by McCallan and Wilcoxon, because the type of germination obtained in the filtrate was approximately the same as that in 0.5 p.p.m. of copper sulphate (0.127 p.p.m. metallic copper).

ACTION OF BORDEAUX WHEN SEPARATED FROM THE SPORES BY FILTER PAPER

In the preceding discussion, it has been brought out that germination was completely inhibited in those tests in which conidia of *Mycosphaerella fragariae* were brought in direct contact with suspensions of Bordeaux mixture, regardless of whether the mixture was freshly prepared, dried, and aged on glass slides, or sprayed and weathered on leaves. However, results of this type do not indicate whether the spores that do not germinate are actually killed or whether germination is merely inhibited.

Clark (4) has called attention to the difference between *point of inhibition of germination* and *death point,* and has emphasized the difficulty of separating the fungus spores from the fungicide, which is necessary if the death point is to be determined. He concluded that inhibition of germination is the important point to consider, as his studies indicated that ". . . if the

germination of the spores be inhibited by a concentration of copper salt, it was merely a question of time when they would be killed." Goldsworthy and Green (5) also distinguished between inhibitory and lethal effects of spray residues.

Some tests were conducted to determine if the spores of *Mycosphaerella fragariae* that failed to germinate when exposed to Bordeaux were actually killed or whether germination was merely inhibited. A small amount of spore suspension (0.5–1.0 cc.) was put in a watch glass, a filter paper of slightly larger diameter than the watch glass was placed over it, then a Bordeaux suspension of the desired dilution was placed on the filter paper. The process was reversed in some of the experiments, *i.e.*, the Bordeaux suspension was placed in the watch glass and the spores on the filter paper. After the desired exposure, the spores were transferred to filter paper in a small funnel, washed thoroughly with tap water, placed on slides in a moist chamber, and examined for germination at the end of 24 hours. Naturally, many of the spores were lost during the process of washing and transferring to the slides, but enough remained for germination counts.

In the beginning, one thickness of filter paper was used, but fearing that some Bordeaux particles might adhere to the paper and get on the slides while the washed spores were being transferred from the filter paper to the slides, 2 layers of filter paper were used in most of the later tests. Where high dilutions of Bordeaux were used (Experiments 8 and 9, Table 7), the method was further modified. The spore suspension was placed on filter paper in a funnel, so that the water would drain and leave the spores within the cone of the filter. The paper was then carefully folded, so as to prevent the escape of the spores, and placed on the bottom of a Syracuse dish. A second piece of filter paper, larger than the inside diameter of the dish, was cupped and placed over the folded paper in the dish, and 10 cc. of the desired dilution of Bordeaux were poured in the dish on the cupped paper. The spores were thus separated from the Bordeaux by 2 thicknesses of filter paper.

Three kinds of checks were provided: (1) Spores in water were washed in the same manner and for the same length of time as were the Bordeaux-exposed spores; (2) Fresh spores in tap water on slides, not washed; (3) Fresh spores in water from the last washing of the Bordeaux-exposed spores. For lack of space, only a few of the results with the checks are included in the summary of the experiments (Table 7), but germination in all cases was high (over 90 per cent).

The method has the serious disadvantage that it does not give a true picture of what happens when there is no partition to separate the spores from the Bordeaux particles. When spores were placed in a suspension of Bordeaux mixture, they have been observed to adhere to the Bordeaux particles, so that there was a very close association between them and the fungicide, an association not attained when a paper partition separates the spores from the Bordeaux particles. Nevertheless, the method proved useful in demonstrating the high sensitiveness of *Mycosphaerella fragariae* conidia

TABLE 7.—*Effect of concentration of Bordeaux and time of exposure on the germination of conidia of Mycosphaerella fragariae when the spores were separated from the Bordeaux particles by means of one or two thicknesses of filter paper. After the desired exposure, the spores were washed on filter paper, transferred to tap water on slides in moist chambers, and examined 24 hours later for germination*

No. and date of experiment	Material[a] and arrangement	Dilution	Period of exposure	Results (percentage germination; average of 4 replications)
1	A	———	17 hrs.	0.0
Jan. 13, 1934	Check[b]	———	——	94.2
	Check[c]	———	——	91.6
2	B	1–21	½ hr.	0.0
Feb. 12, 1934		1–21	1 hr.	0.0
		1–21	2 hrs.	0.0
		1–21	3 hrs.	0.0
	Check[b]	———	——	91.8
	Check[c]	———	——	90.8
3	C	1–21	1½ hrs.	0.0
Feb. 14, 1934		1–21	3 hrs.	0.0
4	D	1–21	½ hr.	0.0
Feb. 14, 1934		1–21	1 hr.	0.0
		1–21	3 hrs.	0.0
5	B	1–20	5 min.	55.5 (germ tubes short)
Feb. 10, 1935		1–30	5 min.	6.1 (" " ")
		1–50	5 min.	11.4 (" " ")
		1–100	5 min.	38.0 (" " ")
		Undiluted	30 min.	0.0
	Check[b]	———	——	93.2 (" " long)
6	E	1–24	15 min.	0.0
Feb. 15, 1935		1–24	30 min.	0.0
		1–44	30 min.	35.0 (germ tubes short, gnarled)
		1–10	60 min.	0.0
7	C	Undiluted	30 min.	0.0
March 4, 1935		1–25	30 min.	8.2[d] (germ tubes as long as those of check)
		1–50	30 min.	0.0 (spores swollen)
		1–100	30 min.	0.0 (" ")
		1–10	5 min.	86.5 (germ tubes shorter than those of check)
		1–20	5 min.	91.5 (germ tubes shorter than those of check)
		1–50	5 min.	95.5 (germ tubes as long as those of check)
		1–20	10 min.	52.0 (only "buds" of germ tubes)
		1–40	10 min.	81.0 (germ tubes long)
		1–80	10 min.	89.0 (" " ")
		1–100	10 min.	85.0 (" " ")
8	C	1–5,000	18 hrs.	63.0 (ave. length of tubes 44.7μ)
March 7, 1935		1–2,000	18 hrs.	51.0 (" " " " 84.0μ)
		1–1,500	18 hrs.	59.5 (" " " " 61.2μ)
		1–1,000	18 hrs.	43.0 (" " " " 62.2μ)
		1–500	18 hrs.	29.0 (" " " " 22.4μ)
	Check[b]	———	——	91.5 (" " " " 97.8μ)

TABLE 7.—(*Continued*)

No. and date of experiment	Material[a] and arrangement	Dilution	Period of exposure	Results (percentage germination; average of 4 replications)
9 March 21, 1935	C	1–500	18 hrs.	54.0 (ave. length of tubes 30.6μ)
		1–400	18 hrs.	61.5 (" " " " 35.7μ)
		1–300	18 hrs.	43.0 (" " " " 43.3μ)
		1–250	18 hrs.	46.0 (" " " " 28.3μ)
		1–200	18 hrs.	38.0 (" " " " 31.5μ)
		1–150	18 hrs.	41.0 (" " " " 22.8μ)
		1–100	18 hrs.	0.0 (spores swollen)
		1–50	18 hrs.	0.0 (no swelling of spores)
		1–25	18 hrs.	0.0 (" " " ")
	Check[b]	———	———	90.5 (ave. length of germ tubes 131.9μ)
	Check[c]	———	———	93.6 (ave. length of germ tubes 142.0μ)

[a] A = Weathered (10 days) Bordeaux residue from leaves in watch glass, separated from spore suspension by filter paper. B = Fresh 4–4–50 Bordeaux separated from spores in watch glass by filter paper. C = Fresh 4–4–50 Bordeaux separated from spores in watch glass by two layers of filter paper. D = 4–4–50 Bordeaux dried and aged on glass slides for 1 week, separated from spores in watch glass by 2 layers of filter paper. E = 4–4–50 Bordeaux, dried and aged on glass slides for 1 year, separated from spores in watch glass by filter paper.

[b] Spores in tap water, washed.

[c] Fresh spores in water from last washing of Bordeaux-exposed spores.

[d] A few of the spores apparently escaped exposure, because there was no gradation in the germination. Of 400 spores examined, 33 were found with germ tubes as long as those of the check; the rest were nongerminated.

to copper toxicity. The results of several experiments with various dilutions of Bordeaux and different periods of exposure are presented in table 7. The outstanding fact brought out by these experiments is that Bordeaux is lethal to the nongerminated conidia, even at relatively short exposures. The spores were killed (or sufficiently injured so that they did not germinate when removed from the fungicide and washed) in every case when exposed to the nondiluted, and to dilute solution of 1–21 Bordeaux for 30 minutes or longer, regardless of whether the Bordeaux was fresh or dried on slides (Experiments 1–7, Table 7). In experiment No. 6 (Table 7), killing of spores occurred at 15 and 30 minutes' exposure to 1–24 dilution of Bordeaux that had been dried and aged on glass slides for one year, and a 30-minute exposure to 1–44 dilution resulted in reduced (35 per cent) and abnormal (short, gnarled germ tubes) germination. In experiment No. 7 (Table 7) no germination occurred after a 30-minute exposure to as high a dilution as 1–100 of freshly prepared 4–4–50 Bordeaux, although the spores were not killed, judging by their swelling after washing. Exposures of 5 to 10 minutes were not lethal, although a certain degree of injury to spores was apparent, even at these short exposures. In one series of tests (Experiment 5, Table 7) injury (as shown by the low percentage of germination and the short germ tubes) resulted by exposing the spores for 5 minutes to dilutions of 1–20, 1–30, 1–50, and 1–100. In another series (Experiment 7, Table 7) the 5-minute exposure had no appreciable effect on the viability of the spores (except for

the shorter germ tubes in the 1–10 and 1–20 dilutions), while a 10-minute exposure to a dilution of 1–20 had a decided effect both on the percentage and type of germination; only 52 per cent of the spores germinated and the germ tubes did not grow beyond the "bud" stage. The tests with the high dilutions of Bordeaux (Experiments 8 and 9, Table 7) were included in order to determine if long exposures to high dilutions are lethal, since it was found earlier that no germination occurred when the spores were in direct contact with a 4–4–50 Bordeaux suspension diluted 1 part in 1,000 parts of water. As seen in table 7, injury resulted from an 18-hour exposure in all dilutions tested (1–25 to 1–5,000) as shown by the reduction in the percentage germination and the length of the germ tubes.

TOXICITY OF SOLUBLE COPPER SALTS

In order to obtain more exact information on the limits of copper toxicity toward *Mycosphaerella fragariae* conidia than was possible when Bordeaux suspensions were used, dilute solutions of copper sulphate ($CuSO_4$ $5H_2O$), copper chloride (anhydrous $CuCl_2$), and copper acetate ($Cu(CH_3CO_2)_2$ H_2O) were prepared and their action on germination was tested. Two types of tests were made: (1) A spore suspension (density of approximately 10 spores per intermediate-power microscopic field) was made in the desired dilution, and a few drops of it placed on slides in a moist chamber and examined for germination 24 hours later. (2) A spore suspension of approximately the same density as above was made in 2 cc. of the desired dilution in a vial and allowed to stand for 24 hours. The spores were then transferred to a folded filter paper in a small funnel, washed with several changes of water, placed in a few drops of water on slides in moist chambers, and examined 24 hours later for germination. The majority of the tests were made with copper sulphate and copper chloride; only a limited number of tests were made with copper acetate. The results of these tests may be summarized as follows:

I. On Slides in the Respective Dilutions, Spores Not Washed. 1. No germination, no swelling of spores in dilutions of 1–250,000 or lower of copper sulphate, 1–500,000 or lower of copper chloride, and 1–400,000 or lower of copper acetate (Fig. 2, F).

2. In dilutions of 1–300,000 to 1–500,000 of copper sulphate, 1–500,000 to 1–1,000,000 copper chloride, and 1–500,000 to 1–800,000 copper acetate, the conidia swelled considerably (Fig. 2, G) but did not germinate.

3. In dilutions of 1–500,000 to 1–1,000,000 of copper sulphate, 1–1,000,000 to 1–3,000,000 of copper chloride, and 1–1,000,000 to 1–2,000,000 of copper acetate, the majority of the conidia produced very short "buds" of germ tubes (Fig. 2, H, I, K, M). No further growth was noted when the slides were reexamined 24 hours later.

4. In dilutions of 1–2,000,000 copper sulphate, 57 per cent of the conidia germinated with short, twisted, curled germ tubes. Some elongation of the germ tubes was noted (some approaching normal growth) when the slides were reexamined 24 hours later. The same type of germination occurred in

1–3,000,000 copper acetate (Fig. 2, O, P), and 1–4,000,000 copper chloride. The toxic action was still evident at as high a dilution as 1–3,000,000 of copper sulphate (0.085 p.p.m. metallic copper). In this dilution, and also in 1–6,000,000 copper chloride (0.078 p.p.m. metallic copper) and 1–4,000,000 copper acetate (0.0796 p.p.m. metallic copper) the germination was high, 76.0 per cent, 77.2 per cent, and 85.0 per cent, respectively, but still not normal, the germ tubes being much shorter than those of the checks and most of them gnarled, twisted, and curled.

5. Normal germination (Fig. 2, L) was obtained in dilutions of 1–4,000,000 copper sulphate (0.0636 p.p.m. metallic copper). Dilutions of copper chloride higher than 1–6,000,000 and of copper acetate higher than 1–4,000,000 were not used; thus, the limits at which normal germination occurs in these compounds were not determined.

II. Spores Washed After a 24-hour Exposure to the Copper Solution. Only copper sulphate in dilutions of 1–100,000 to 1–2,000,000 was used in this experiment and only 2 separate tests were made. No germination and no swelling of spores occurred in dilutions of 1–500,000 or lower. In one test in which acidified tap water was used in making the dilutions, no swelling of spores occurred in 1–600,000 dilution, while in the 1–1,000,000 dilution most of the spores were swollen but not germinated. In the second test, in which the dilutions were made with rain water, 14.5 per cent of the spores were merely swollen and 10.5 per cent were swollen and had short "buds" of germ tubes in the 1–600,000 dilution. In the 1–1,000,000 dilution 11.5 per cent of the spores were swollen and had short "buds," and 20.0 per cent had short gnarled, curled germ tubes. In the 1–2,000,000 dilution, the type of germination was approximately the same in both tests. There was an average germination of 82.0 per cent, but the germ tubes varied in length from mere "buds" to short, gnarled, curled germ tubes. Many of the spores had multiple germ tubes (germination from the ends and from the sides); the germination at this high dilution of copper sulphate was decidedly abnormal. It would appear from these results that washing did not remove the toxic action of copper. Goldsworthy and Green (5) have shown that, once the copper has been absorbed by the cell, no amount of washing will remove it.

As seen from the results of the tests with soluble copper salts, the limits of toxicity of the different dilutions are not sharply defined. For example, there was very little difference in the appearance of the spores in dilutions of copper sulphate of 1–500,000, 1–600,000, 1–800,000, and 1–1,000,000. In all these dilutions, the spores swelled greatly and produced short "buds" of germ tubes but made no further growth. It is a moot question whether to consider as the critical limit of toxicity (1) the highest dilution in which the germination does not proceed beyond the "bud" stage of germ-tube formation, (2) the one in which abnormal germination (short, gnarled, curled germ tubes) occurs, or (3) one just below the highest dilution in which the germination is normal. If the third is taken as the critical point, then the limit of copper toxicity toward conidia of *Mycosphaerella fragariae* lies somewhere

between dilutions of copper sulphate of 1–3,000,000 (0.085 p.p.m. metallic copper) and 1–4,000,000 (0.0636 p.p.m. metallic copper). Infection most likely will not take place if the concentration of available copper on the leaves is such as to inhibit germination beyond the "bud" stage, and it is doubtful that it will occur under conditions in which germination is abnormal.

COMPARISON BETWEEN CONIDIA OF MYCOSPHAERELLA FRAGARIAE AND THOSE OF OTHER FUNGI IN THEIR SENSITIVENESS TO COPPER TOXICITY

The extreme sensitivity of *Mycosphaerella* conidia to copper toxicity may be illustrated by comparing it with that of spores of some other fungi. In one of the experiments with copper chloride, conidia of *Botrytis* sp. (probably *B. cinerea,* taken from a decaying stem of lupine) were included in the 1–300,000 and 1–500,000 series of dilution. Germination of 52.0 per cent and 56.0 per cent, respectively, was obtained in these dilutions (93.5 per cent in tap water). The germ tubes were also long and apparently normal. The *Mycosphaerella fragariae* conidia, on the other hand, not only did not germinate in these dilutions, but, from all appearances (failure to swell and coagulation of their protoplasm) were actually killed in the nongerminated stage.

Spores of other fungi occurred occasionally on slides, and it was noted that they would germinate in dilutions in which *Mycosphaerella* conidia would not. For example, in one experiment 4 conidia of *Cladosporium* sp. were found on one slide in the 1–1,000,000 copper sulphate dilution series. These had germinated well with long, branched germ tubes, while the *Mycosphaerella* conidia were only swollen, with mere "buds" of germ tubes. The same thing occasionally was observed with spores of *Alternaria* sp. In figure 2, N, is shown an *Alternaria* spore germinating well in 1–2,000,000 copper acetate, while a *Mycosphaerella* conidium, next to it, did not germinate beyond the "bud" stage. Bacterial growth, also, occurred on the slides in dilutions of copper, which inhibited germination of *Mycosphaerella* conidia. The copper-sensitivity of *Mycosphaerella* conidia, under laboratory conditions, surpasses that of algae. Moore and Kellerman (11, 12) found that Spirogyra, one of the most copper-sensitive algae, made good growth in 1 part of copper sulphate in 200,000 parts of water in the laboratory, while 1 part of copper sulphate in 25,000,000 parts of water was sufficient to kill this alga under natural conditions in water reservoirs.

DISCUSSION

It is evident from the results of this investigation that the explanation for the effectiveness of Bordeaux spray against the strawberry leaf spot lies in the extreme sensitiveness of the conidia of the causative fungus to copper toxicity. It has been shown previously that infection occurs primarily through the under leaf surface. However, because of the low, flat habit of growth of the strawberry plant, and the system of spraying practiced in

Louisiana (small, low-pressure sprayers are used), very little of the spray material reaches the under leaf surface. The action of Bordeaux, therefore, cannot be considered protective in the sense that it protects the sprayed surface from infection. It may more properly be considered as eradicative, since it has been shown that the nongerminated spores are killed when they come in contact with the spray residue. So sensitive are the conidia of *Mycosphaerella fragariae* to the toxic action of copper that germination was completely inhibited when the spores were exposed to fresh or dried Bordeaux for as short a period as 30 minutes and then removed from the sphere of influence of the fungicide and washed. A certain degree of toxicity was apparent even at shorter exposures (Table 7). In tests with soluble copper salts, normal germination was not obtained even in as high a dilution as 0.33 p.p.m. of copper sulphate (0.085 p.p.m. of metallic copper).

It is believed that the results obtained justify the following conclusions relative to the mode of action of Bordeaux spray in controlling Mycosphaerella leaf spot of strawberries: (1) The spray greatly inhibits sporulation on the infected leaves, especially on the upper leaf surface. This reduces considerably the amount of potential inoculum. (2) Conidia that come in contact with a sprayed surface (leaves, or the pine-needle mulch) are killed if sufficient moisture be present to wet the spray residue. (3) Some of the copper that comes in solution from the spray residue may diffuse through the dead tissue of the older spots, or run over the margin of the leaf and reach and kill some of the conidia on the under surface. (4) The limited amount of spray material that may reach the under surfaces of some of the noninfected leaves is probably sufficient, considering the extreme sensitiveness of the spores to copper, to protect them from infection. (5) The spray residue on the upper leaf surface prevents the small amount of infection that has been shown (13) to take place through the limited number of stomata that occur on the upper leaf surface. In the last two instances the action of Bordeaux is protective.

SUMMARY

The purpose of the present investigation was to determine the mode of action of Bordeaux spray on *Mycosphaerella fragariae*.

The spray does not affect the fungus mycelium within the host tissue.

Sporulation on the upper surface of infected leaves is greatly inhibited by the spray. Between 7 and 10 times as many spores were found on nonsprayed as on sprayed leaves. This reduces considerably the amount of potential inoculum.

The conidia were found to be extremely sensitive to copper toxicity. In direct contact with freshly prepared 4–4–50 Bordeaux mixture, germination was completely inhibited by as high a dilution as one part of Bordeaux in 1,000 parts of water. A certain degree of toxicity (as judged by the length of the germ tubes) was evident in dilutions of 1–10,000 and 1–20,000 of 4–4–50 Bordeaux.

No germination was obtained when spores were brought in contact with Bordeaux dried and aged on glass slides (one week to over a year) or with Bordeaux weathered on leaves in the field for 50 days. The nongerminated spores appeared dead (coagulated protoplasm) at the end of 24 hours.

A 24-hour exposure to spores in water on sprayed strawberry leaves, after 10 days' weathering in the field, was lethal.

The spores failed to germinate in the supernatant clear liquid of fresh 4–4–50 Bordeaux. In the supernatant liquid of Bordeaux that had been dried on glass the germination was abnormal (short, curled, gnarled germ tubes).

Lime was not toxic. Excellent germination was obtained in 1 per cent lime smears on slides.

When the conidia were separated from the Bordeaux mixture by a partition of filter paper, it was found that they were either killed or permanently injured by an exposure to 30 minutes or longer, for they failed to germinate when removed from the sphere of influence of the fungicide and washed.

When the spore suspension was connected to dry Bordeaux smears on glass slides by means of narrow water bridges, no germination occurred at distances of 1, 2, or 3 mm. At distances of 5, 10, and 16 mm., germination was initiated, the length of the germ tubes being in direct proportion to the distance, but growth soon stopped. It was evident that germination occurred before sufficient copper had diffused through the water bridge.

Solutions of 1–250,000 copper sulphate, 1–500,000 copper chloride, and 1–400,000 copper acetate were lethal to the nongerminated conidia. In higher dilutions (1–500,000 to 1–1,000,000 of copper sulphate, for example) the conidia germinated with very short "buds" of germ tubes, but no further growth occurred. Normal germination, analogous to that of the checks, was obtained in dilutions of 1–4,000,000 of copper sulphate.

It is concluded that the action of Bordeaux spray in controlling the strawberry leaf spot is mainly eradicative rather than protective in character. The spray (1) inhibits sporulation to a large extent, and (2) kills the nongerminated spores that may come in contact with it. A minor degree of protective action also is recognized.

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THE INFLUENCE OF FOUR MOSAIC DISEASES ON THE PLASTID PIGMENTS AND CHLOROPHYLLASE IN TOBACCO LEAVES[1]

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INTRODUCTION

The mosaic viruses are characterized by their ability to reduce the intensity of the green color in irregular zones in the leaves and sometimes the stems, floral parts and fruits of mosaic-susceptible plants. These irregular zones in contrast to those that remain essentially a normal green produce a mosaic pattern.

Microscopic observations and analyses made by several investigators (1, 2, 3) working with tobacco mosaic have indicated that the chlorophyll content is reduced in the light-green zones. These studies presumably were confined to the common mosaic, which usually induces many light-green areas on the foliage. In the present studies, 4 distinct mosaics, differing in their color or pattern characteristics, were used; one, however, only to a limited extent. In addition to studying the chlorophyll, attention was given to the carotene and xanthophyll and to the enzyme chlorophyllase. A brief abstract of the results has been published (7).

MATERIALS

All of the analyses were carried out with Wisconsin-Havana seed tobacco. The 4 viruses and the mosaics they induce are described in considerable detail

[1] All of the studies dealing with the pigments and chlorophyllase were conducted by the senior writer. The studies relating to the general pathological properties and other properties of the viruses presented in tables 1 and 2 and as set forth in the sections dealing with the detailed descriptions of symptoms on tobacco were conducted by the junior writer or under his immediate supervision. All work was done at the Arlington Experiment Farm, Arlington, Va.

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TABLE 1.—*Types of symptoms induced by four distinct mosaic viruses at the temperatures and on the test plants indicated. All solanaceous species were inoculated after the seedling stage when the plants were well established in clay pots. Culture was in greenhouse or chambers with natural daylight and photoperiods*

Test plants	Common mosaic virus	Yellow mosaic virus (Type A)	Mild dark-green mosaic virus	Mild mosaic virus
Nicotiana tabacum L.,	18° to 37° C. *A*[a]	13° to 36° C.[a] *A*[f]	18° to 34° C. *A*	21° to 35° C. *A*
Var. Wisconsin-Havana seed	29° to 37° C. *D*	25° to 36° C. *D*		
Nicotiana tabacum L.,				
Var. Ambalema	21° to 37° C. *F*[c]	21° to 37° C. *F*[c]	21° to 37° C. *F*	21° to 35° C. *A*
Lycopersicum esculentum Mill.,				
Var. Bonny Best	21° to 27° C. *A*	21° to 29° C. *A*	18° to 34° C. *G*	21° to 35° C. *A*
Nicotiana sylvestris		18° to 24° C. *C*	18° to 24° C. *C, E*	18° to 32° C. *B*
Spegaz. and Comes	21° to 32° C. *A*	31° to 34° C. *D, E*	31° to 32° C. *A, D*	
			C rarely	
Nicotiana langsdorffii Schrank	15° to 34° C. *C, E*	21° to 34° C. *C, E*	18° to 24° C. *C, E*	18° to 32° C. *A*
	D rarely	*D* rarely	31° to 32° C. *D, E*	
Nicotiana rustica L.	15° to 24° C. *C*	15° to 24° C. *C*	15° to 32° C. *C*[d]	21° to 32° C. *A*[e]
	31° to 34° C. *D, E*	31° to 34° C. *D, E*		
Nicotiana glutinosa L.	15° to 24° C. *C*	15° to 24° C. *C*	15° to 24° C. *C*	21° to 32° C. *A*[e]
	31° to 34° C. *D, E*	31° to 34° C. *D, E*	31° to 32° C. *C, D, E*	
Capsicum frutescens L.	15° to 24° C. *C*	15° to 24° C. *C*	15° to 24° C. *C*	21° to 34° C. *A*[e]
	31° to 34° C. *D, E*	31° to 34° C. *D*	31° to 34° C. *D, E*	
		E rarely		
Solanum tuberosum L.				
Var. Green Mountain				
(seedling)				15° to 24° C. *A, D*
Phaseolus vulgaris L.				
Var. Scotia	18° to 37° C. *C*	18° to 37° C. *C*	15° to 37° C. *G*	15° to 37° C. *G*
Cucumis sativus L.				
Var. White Spine	21° to 31° C. *G*	21° to 31° C. *G*	21° to 34° C. *G*	21° to 34° C. *G*

[a] The wide ranges of temperature cited do not signify that the temperatures fluctuated between the degrees recorded, but tests were made at several points within these ranges.

[b] At 37° C. mottling appears on 3 to 4 leaves, but subsequent leaves usually show no mottling; near 15° C. mottling is light green.

[c] Virus systemic, but mottling is usually absent in the strain of Ambalema used.

[d] Virus systemic, but erratic, inducing necrotic lesions occasionally on new leaves that are not wiped.

[e] Virus not systemic and no local symptoms at 15° C.

[f] The capital letters in the columns refer to the type of symptoms expressed as indicated below by the corresponding letters. The temperature ranges cited in the columns indicate actual tests and not necessarily absolute limits.

A. Virus systemic, inducing mosaic mottling not lethal.
B. Virus systemic, inducing severe veinbanding, which sometimes produces a mottled effect, not lethal.
C. Virus induces local necrotic lesions on mature and nearly mature leaves when inoculated by wiping.
D. Virus induces local chlorotic spots on mature and nearly mature leaves when inoculated by wiping.
E. Virus systemic, sometimes induces mottling on new foliage, induces necrosis and premature death of part or all of the plant.
F. Very resistant.
G. Immune.

in order that they may be compared and contrasted with some degree of exactness. In table 1 the characteristic symptoms on several plant species are recorded with the temperature ranges over which they were observed. This method was necessary, since temperature frequently modifies symptoms to a degree equaling the modifying influences of certain plant species, and to a degree that confuses certain distinct viruses.

The simple properties of the viruses outside of the plant are listed in table 2.

TABLE 2.—*Notes on the properties of the viruses outside of the living plant*

	Tobacco common-mosaic virus	Tobacco yellow-mosaic virus (Type A)	Mild dark-green mosaic virus	Mild-mosaic virus
Inactivating temperature of fresh non-diluted virus in the plant extract with 10 min. exposure	Near 90° C.	Near 90° C.	Near 86° C.	Near 56° C.
Survival at room temperature (Dry leaf tissue)	Many years	Many years	Many years	3 to 10 days
Survival at $+5 \pm 2°$ C. (Plant extract)	do	do	do	20 to 30 days
Survival at $-8 \pm 1.5°$ C. (Plant extract)	do	do	do	50 to 60 days
Survival at $-17 \pm 1°$ C. (Plant extract)	do	do	do	Over 174 days
Dilution end point in water[a]	10^{-5} to 10^{-6}	10^{-5} to 10^{-6}	5×10^{-4} to 10^{-3}	5×10^{-2} to 10^{-5}

[a] In the case of each virus the fresh plant extract was diluted in distilled water and tested by means of systemic infection in tobacco or *Nicotiana sylvestris*.

The symptoms induced by each virus on Wisconsin-Havana seed tobacco cultured in glass chambers and greenhouses at 22° to 27° C. with natural daylight and photoperiods are given in detail in the following paragraphs in order that similarities and differences may be readily determined from comparable material. This cannot be done satisfactorily on a basis of existing literature.

Common Mosaic

At temperatures near 27° C. the virus induces local chlorotic spots on the young leaves when inoculated by wiping. Vein clearing in the new leaves is the first sign of systemic infection, followed by light-green patches on the subsequent leaves. Vein clearing does not persist. The light-green areas usually involve more than 50 per cent of the leaf area on mature and nearly mature leaves; the darker or normal green areas are broken up into many patches. From 3 to 6 of the apical leaves usually develop an upward roll from 1 to 2 mm. wide along the margins, especially near the tips. In addition to this marginal roll, the leaf as a whole may curve downward producing an

"inverted-spoon" effect. Also, the surface of the leaves may exhibit puckering and the margins may be irregular. The virus mutates (4, 5) in local zones in the plant, the yellow-mosaic mutants inducing occasional small yellow spots on the leaves. This virus has been used in previous studies by the junior writer (4). It was obtained originally from James Johnson and, presumably, is the virus which he later designated *tobacco-mosaic virus 1*.

Yellow Mosaic (Type A)

Local chlorotic spots and vein clearing appear as with common mosaic. The mosaic pattern is creamy white, yellow or yellowish green, and occurs on foliage, stem, and fruits. The mosaic patterns are similar in size and shape to those induced by the common-mosaic virus. Upward rolling of the leaf margins, leaf puckering and marginal irregularity are more evident than in common mosaic. The virus originated as a mutant from the common-mosaic virus used in these studies (4).

Mild Dark-Green Mosaic

Local chlorotic spots and vein clearing appear as with common mosaic. After vein clearing the subsequent young leaves—usually 3 to 5—show upward marginal rolling and stunting, but usually little or no mottling appears. Mottling on subsequent leaves—usually 3 to 5—consists of very small light-green areas, which are increasingly fewer but larger on each successive leaf, until the symptoms resemble the mottling induced on tobacco by Johnson's *cucumber virus 1* supplied by S. P. Doolittle. This stage may be followed by leaves that develop the very small light-green mottled areas.

When mottling is most evident the areas tend to be fewer and larger than is the case with common mosaic. Plants with mild dark-green mosaic have a darker green appearance and frequently they show more leaf puckering and more of the "inverted spoon" effect than plants having the common mosaic or the yellow mosaic.

This virus was collected in *Nicotiana glauca* R. Grah. on the island of Grand Canary. It has never given evidence of producing yellow-mosaic mutants in the junior writer's tests (4).

Mild Mosaic

No local chlorotic spots. Vein clearing as with common mosaic. The subsequent 4 to 6 leaves show diffuse light-green patches, some of which contain darker green islands. Subsequent leaves tend to show small definite light-green mottlings at the tips, at the base or along the margins. These signs tend to continue in 4 to 6 of the apical leaves throughout the life of the plant. As these leaves develop the light green approaches more nearly normal green, producing a diffuse light-green patching that may involve the entire leaf. These patches are not conspicuous and frequently disappear. As leaves approach senility, small dark-green mottlings sometimes appear.

The shape of the leaves remains essentially normal, showing little or no

marginal rolling and no puckering. This mosaic is not so mild on tobacco or on *Nicotiana sylvestris* as is the potato veinbanding mosaic, which was supplied to the junior writer by E. S. Schultz. However, it is less severe on tobacco and on *N. sylvestris* than is the potato "Y" mosaic supplied by T. P. Dykstra. On *N. sylvestris* the 3 viruses induce veinbanding, which persists throughout the life of the plants; but this symptom is little more than a pronounced vein clearing in the case of the true veinbanding type, whereas, in the case of the mild mosaic, chlorosis is more pronounced in the tissue adjacent to the veins and veinlets. The "Y" virus induces severe chlorosis and some leaf deformation on *N. sylvestris*.

Although this mosaic is referred to tentatively as mild mosaic, further studies may show it to be a strain of potato "Y" mosaic or of veinbanding mosaic.

Mild mosaic has given no evidence of mutation in these studies. The virus was collected by the junior writer on plants of growing tobacco at Ephrata, Pennsylvania.

A Condensed Scheme for Differentiating the Four Viruses

The virus of mild-mosaic is inactivated in 10 minutes near 56° C., whereas the inactivation temperatures are considerably higher for the other viruses. This virus does not induce necrotic lesions on any of the plants tested.

The virus of mild dark-green mosaic does not induce necrotic lesions on beans nor does it go to tomato, whereas the viruses of common mosaic and yellow mosaic do.

The virus of yellow mosaic induces local necrotic lesions but no mottling on *Nicotiana sylvestris* at 22° C., whereas the virus of common mosaic induces mottling but no necrotic lesions.

METHODS

Tobacco was selected as the plant with which to work because it is subject to virus diseases of several different types and its large leaves provide ideal material for pigment analysis.

The plants were grown in 8-inch pots in a greenhouse at Arlington Farm. The pots were grouped centrally in the house and partially shaded to provide uniform conditions of light and to prevent sunscald of plants affected with yellow mosaic. Aside from routine care the plants were watered once each week with a ½ per cent solution of calcium nitrate to prevent premature yellowing of basal leaves.

Because of the extreme stunting effect of yellow mosaic, it was necessary to grow at least 10 plants of this type against 6 each of all other types, including healthy plants, to provide sufficient material for pigment analysis. All plants used in a series were selected for uniformity and inoculated when about 6 inches high.

The first pigment determinations were made when the healthy plants were about 18 inches tall. The leaves selected for pigment analysis were picked

at a level about one-third the distance from the tip toward the base of the plant. Leaves at this level were almost completely expanded and showed no symptoms of chlorophyll deficiency due to aging or nutritional causes.

The leaf samples were collected in the morning, usually before 9:00 a. m. The plants were watered heavily the night before to insure uniform turgidity when the samples were taken. Two 10-gram samples—one for pigment analysis, the other for dry weight determinations—were weighed immediately from opposite halves of the same leaves. Midribs and large veins were excluded from the samples by stripping out the interveinal areas by hand.

The method of pigment analysis first used was that of Willstätter as modified by Schertz (8). In the present studies it was found that considerable time could be saved, particularly in the separation of the yellow pigments, by altering the method. Details of the modified procedure have been published (6) and will not be reviewed in detail in this paper.

In accordance with the method cited, all measurements of pigment content were made colorimetrically. Chlorophyll was measured as chlorophyllin; the yellow pigments, carotene and xanthophyll, were measured without chemical alteration. In the absence of fixed color standards, the freshly extracted pigments of healthy tobacco plants were used as standards with which to obtain relative values for each of the mosaic types.

Willstätter's method (9) was used for the determination of chlorophyllase. In making chlorophyllase comparisons involving healthy tobacco and the different mosaic types, errors due to differences in time and environment were avoided by simultaneous extractions of the several samples as in the case of pigment analyses. Each important step in the extraction of a group of samples was completed in a single work day. Details of the method are given below.

The finely ground solids remaining after the complete extraction of the plastid pigments were used as the source of material for the determination of chlorophyllase activity. One-half gram samples of the air-dried solids were placed in 200 cc. Erlenmeyer flasks together with 100 cc. aliquots of freshly extracted chlorophyll. The chlorophyll solution was prepared for each test by extracting 500 grams of fresh, finely ground, healthy tobacco leaves with sufficient amounts of 85 to 90 per cent acetone solution to deliver slightly in excess of 600 cc. of the extract. The filtered extract was divided into aliquots by running it into 100 cc. volumetric flasks from a 600 cc. separatory funnel. The 100 cc. aliquots, after being added to the solids in the Erlenmeyer flasks, were diluted with sufficient water to make a 66 per cent solution (volume basis) of acetone. The amount of water to be added was determined by means of a hydrometer prior to dividing the sample.

After the solution was diluted the flasks were stoppered and set aside in the dark at room temperatures for periods of from 12 to 24 hours. By using some form of constant shaking apparatus the time interval can be shortened. Under the conditions specified, however, shorter intervals than 12 hours in the case of tobacco did not allow for a sufficient conversion of chlorophyll for

accurate colorimetric measurement; longer intervals tended to mask differences in chlorophyllase activities and were, therefore, avoided.

The combined solids and pigment extracts were poured into a glass Büchner funnel and completely extracted with a minimum amount of pure acetone. The solids were then washed with approximately 150 cc. of diethyl ether. In this process a wide-mouth separatory funnel was used in the dual capacity of suction flask and separatory funnel, thus eliminating the need of transferring the solution of the pigments after its separation from the solids.

The combined ether-acetone extracts were shaken vigorously in the separatory funnel to transfer the pigments completely to the ether layer. The ether solution of the pigments was then freed of acetone by careful but thorough washing with distilled water.

After the complete removal of the acetone, the altered chlorophyll was extracted with 0.02N KOH in distilled water to which a few cc. of methyl alcohol had been added. Fifty cc. of the KOH solution were used in the first extraction. The ether extract of the pigments and the aqueous KOH were shaken together vigorously for periods of very short duration. Complete separation of the two solutions was permitted between each shaking. Prolonged vigorous shaking may result in the formation of emulsions that are difficult to "crack."

When the separation of the altered chlorophyll from the unaltered fraction remaining in the ether was complete the dilute KOH extract was filtered through filter paper into a 100 cc. volumetric flask. The ether solution of the pigments was washed several times with a few cc. of KOH solution to remove the last traces of altered chlorophyll. The combined washings were then made up to volume with methyl alcohol. Methyl alcohol was used instead of dilute KOH solution because it has a clarifying effect which facilitates colorimetric analysis. The altered chlorophyll solution is unstable and was, therefore, measured immediately.

The unaltered chlorophyll remaining in the diethyl ether after the removal of the altered fraction may be saponified at this stage and measured as chlorophyllin as a check against the values obtained for altered chlorophyll. Except to determine its feasibility, this procedure was not followed in the present work.

EXPERIMENTAL RESULTS

Plastid Pigments

The plastid pigment data given in table 5 are based on green weight determinations. Green weight was found to coincide fairly closely with area, *i.e.*, when leaf samples were collected on a constant-area basis, their green weights roughly approximated a constant. This same relationship existed for dry weights of healthy plants and all mosaic types not causing severe leaf distortions. The dry weight of yellow mosaic samples, however, was consistently low (Table 3) and pigment comparisons made on a dry weight basis were completely out of line with the visible evidence of chlorosis. Therefore, except in table 4, dry weight comparisons have been omitted.

TABLE 3.—*Weight in grams of 7058 sq. mm. area of tobacco leaf samples from healthy tobacco, common-mosaic, yellow-mosaic, and mild dark-green mosaic-diseased plants*

Sample No.	Healthy		Common mosaic		Yellow mosaic		Mild dark-green mosaic		Month sampled
	Green	Dry	Green	Dry	Green	Dry	Green	Dry	
1	6.54	.61	6.32	.68	7.01	.....	6.57	......	January
2	6.66	.75	6.69	.74	6.70	.54	6.48	.70	"
3	6.96	.83	6.46	.70	6.65	.62	6.36	.75	"
4	6.63	.81	6.29	.68	6.74	.57	6.50	.71	"
5	7.52	.67	....	.....	7.12	.61	7.27	.77	"
6	7.55	.82	7.61	.92	7.27	.64	6.93	.88	February
Average	6.98	.75	6.67	.74	6.92	.60	6.69	.76	
Averages in terms of percentage	100	100	95.5	98.6	99.1	80.0	95.8	101.3	

TABLE 4.—*Relative chlorophyll content of leaves of healthy and mosaic tobacco plants, determined colorimetrically, in terms of depth of solution in millimeters*

Sample No.	Healthy plants	Green-weight basis		Dry-weight basis		Month sampled
		Common mosaic	Yellow mosaic	Common mosaic	Yellow mosaic	
1	10.00	10.24	15.32	13.31	15.32	February
2	10.00	13.20	21.68	17.66	22.11	"
3	10.00	13.36	23.41	....	15.33	March
4	10.00	12.70	23.68	12.70	16.83	"
5	10.00	12.50	20.96	12.68	15.02	"
6	10.00	11.34	15.52	11.08	11.30	"
7	10.00	10.96	18.95	12.08	13.45	April
Average	10.00	12.04	19.92	13.25	15.62	
Averages in terms of percentage	100	83.0	50.2	75.4	64.0	

Typical values for 7 separate pigment extractions, all made during the life of a plant series over a period approximating 2 months, are given in table 4. In this series comparisons are made, both on a green weight and dry weight basis, between healthy plants and those having common mosaic, and yellow mosaic. The values recorded represent averages in millimeters of solution of from 4 to 10 (usually 5) separate readings on the colorimeter and, as recorded, are inversely proportional to concentration.

In table 5 colorimetric values are given that indicate the relative chlorophyll, carotene, and xanthophyll contents of healthy tobacco plants and similar plants affected with common mosaic, yellow mosaic, mild dark-green mosaic and mild mosaic. The plastid-pigment extracts from the healthy plants in each series were used as the standards of comparison. The values have been averaged where more than one analysis was involved. For example, in series 1, table 5, the values given are averages of 4 separate pigment analyses. Averages for the entire series are given at the bottom of the table on both an actual and a percentage basis.

In table 4, values under samples 1, 6, and 7 are considered to be atypical and are, therefore, excluded from the general averages in later tables. In

TABLE 5.—*Relative chlorophyll, carotene, and xanthophyll content of tobacco leaves affected with four types of mosaic (determined colorimetrically in terms of depth of solution in mm., compared with a depth of 10 mm. of solution from healthy plants)*

No. of analyses	Chlorophyll				Carotene				Xanthophyll				Month sampled
	Mosaic				Mosaic				Mosaic				
	Common	Yellow	Mild dark-green	Mild	Common	Yellow	Mild dark-green	Mild	Common	Yellow	Mild dark-green	Mild	
4	12.94	22.42			12.76	23.12			12.72	20.62			Feb.–March
2	14.29	25.44	11.11		15.13	26.93	11.60		14.20	21.09	11.61		May
1	12.46	20.26	10.75		12.74	18.72	10.70		13.14	18.72	11.10		July
1[a]	12.96	24.35			12.36	27.82			13.04	20.14			Sept.–Oct.
1[a]	12.22	18.84	11.04		13.96	24.36	10.56		13.10	22.98	10.38		Dec.
2	15.44	27.35	12.99	13.85	15.45	31.84	13.26	14.34	14.75	24.44	13.52	13.29	March
Average	13.38	23.11	11.47	13.85	13.75	25.47	11.53	14.34	13.49	21.33	11.65	13.29	
Average in %	74.7	43.3	87.2	72.2	72.8	39.3	86.7	69.7	74.1	46.9	85.8	75.2	

1[a] = Two chlorophyll analyses.

explanation, it should be stated that sample 1 was collected early in the life of the plants before the full effects of mosaic were in evidence. Samples 6 and 7 were collected when the plants were approaching maturity and the symptoms of mosaic were becoming masked. In later work fewer pigment analyses in any one plant series were made and the extremes cited were thus avoided.

The chlorophyll, carotene, and xanthophyll content of all mosaic types (Table 5) falls below that of the controls and, though there is a considerable fluctuation in the values from one series of tests to the next, the different mosaics hold their relative positions in all the tests, with the possible exception of mild mosaic. The chlorophyll and carotene determinations place this type between common mosaic and yellow mosaic. On a basis of xanthophyll content, however, mild mosaic lies between mild dark-green mosaic and common mosaic. The actual differences in values are slight and may be of no significance. An insufficient number of analyses were made of mild mosaic leaf tissue to give the values much weight but it is included for purposes of record.

On the average, reduction in the amount of chlorophyll is shown to be accompanied by an approximately proportional drop in 2 yellow pigments, carotene and xanthophyll. The yellowing of tobacco as a result of mosaic thus is clearly due to the partial elimination of chlorophyll as a masking agent rather than to an increase in yellow pigmentation as might be inferred from visual inspection of the plants.

Chlorophyllase Studies

Data pertaining to the measurement of the chlorophyllase activities of healthy and mosaic tobacco leaf tissues are cited in tables 6 to 9, inclusive.

TABLE 6.—*Relative chlorophyllase activity of leaf tissue of healthy and mosaic tobacco plants, as measured colorimetrically, in terms of depth of solution in millimeters*

Series No.	Healthy plants	Common mosaic	Yellow mosaic	Mild dark-green mosaic	Mild mosaic	Number of assays	Month
1	10.00	9.05	3.46	7.40	....	5	February to March
2	10.00	7.76	3.25	6.40	....	2	May
3	10.00	11.39	8.35	9.83	....	1	July
4	10.00	12.18	5.41	11.18	....	2	December
5	10.00	12.68	6.25	7.42	7.98	1	March
Average	10.00	10.61	5.35	8.45	7.98	.....	
Average in terms of percentage	100.00	94.3	186.9	118.5	125.3		

The values in table 6 show the relative chlorophyllase activity of the ground and extracted leaf tissues of healthy and mosaic tobacco. With the exception of common mosaic, the chlorophyllase content of mosaic plants averages

TABLE 7.—*Relative chlorophyllase and plastid-pigment content of healthy and mosaic tobacco. Values are averages from tables 5 and 6*

Enzyme or pigment	Healthy plants	Common mosaic	Yellow mosaic	Mild dark-green mosaic	Mild mosaic
Chlorophyllase	100	94.3	186.9	118.5	125.3
Chlorophyll	100	74.7	43.3	87.2	72.2
Carotene	100	72.8	39.3	86.7	69.7
Xanthophyll	100	74.1	46.9	85.8	75.2

TABLE 8.—*Relative chlorophyllase activity and plastid pigment content of the green and yellow areas of yellow mosaic leaves*

	Green	Yellow
Chlorophyllase	100	123
Chlorophyll	100	67
Carotene	100	86
Xanthophyll	100	98

TABLE 9.—*Relative chlorophyllase activity and pigment content of leaves from near the top, middle, and base of healthy tobacco plants*

	Top	Middle	Bottom
Chlorophyllase	100	52	22
Chlorophyll	100	53	20
Carotene	100	69	20
Xanthophyll	100	61	20

appreciably higher than that of healthy plants. The chlorophyllase activity of yellow mosaic averages about double that of healthy leaf tissue. This is particularly significant when compared with the pigment data in table 5, which show that the chlorophyll content of yellow-mosaic leaves is about one-half that of healthy leaves. The approximate halving of the chlorophyll content by yellow mosaic was, therefore, associated with about a twofold increase in chlorophyllase activity.

Table 7 is compiled from tables 5 and 6 to facilitate comparisons between plastid-pigment and chlorophyllase values. The values for chlorophyllase, chlorophyll, carotene, and xanthophyll in the case of mild mosaic are based on a single assay. All other values are averages of 11 to 13 assays as indicated in the table. With the exception of common mosaic the chlorophyllase activity of the mosaic plants is higher than that of healthy plants. The pigment content of all mosaic types, in contrast, is lower than the controls.

The data in table 8 indicate the relative chlorophyllase activity and plastid-pigment contents of the green and yellow tissues of yellow-mosaic leaves. It will be noted that the yellow tissues contain considerably less chlorophyll, somewhat less carotene, and about the same amount of xanthophyll. In contrast, though of lower chlorophyll content, the yellow leaf tissues are higher in chlorophyllase activity than are the green tissues.

The values given in table 9 indicate the relative chlorophyllase activity and pigment content of leaves from the top, middle and base of healthy tobacco plants. Basal leaves were pale green to yellow. Chlorophyllase activity in this case is almost directly proportional to pigment content. As pigment content falls off, chlorophyllase activity slows down. A halving of the chlorophyll content—middle leaves, compared with the top—is accompanied by an approximate halving in chlorophyllase activity.

The relative chlorophyllase content of different tissues from plants with yellow mosaic were as follows: leaf tissue 100, cortex 14, pith and phloem 9, and roots 9. The amount of change in the chlorophyll extract produced by root and pith tissues was almost negligible. Both root and pith were devoid of chlorophyll, the phloem tissues were very low in chlorophyll, while the leaves contained approximately one-half as much chlorophyll as did the healthy plants.

DISCUSSION AND CONCLUSION

Reduction in chlorophyll content by mosaic was accompanied by an approximately proportional drop in xanthophyll and carotene. The yellowing of tobacco is thus due to the partial elimination of chlorophyll as a masking agent rather than to any actual increase in yellow pigmentation.

These results would seem to differ from those of Elmer (3) who, though reporting a reduction in xanthophyll content, found an approximate doubling of the carotene content in both the dark-green and light-green areas of tobacco mosaic. Presumably, Elmer worked with the common mosaic of tobacco but the discrepancy between his findings and those here reported suggests the possibility that he may have used a virus isolate quite different from the common mosaic used by the writers. This possibility is of interest, in view of the fact that none of the four virus types used in the present studies increased the carotene content. It is possible also that environmental factors influence plastid-pigment production with reference to a given virus.

Owing to the difficulties encountered in word descriptions and to the high cost of colored lithographs it appears that plastid-pigment analyses offer one of the best means for describing the degrees of green and yellow colorations manifested by different mosaics. Some consideration has been given to spectroscopic methods for analyzing the colors on the leaf surface, but several factors of error must be studied before the value of this method is known.

With the possible exception of common mosaic of tobacco, all the mosaics studied tended to increase the chlorophyllase activity of tobacco leaf tissues. The increase in chlorophyllase activity in each case was accompanied by a decrease in the chlorophyll content of the tissues.

In direct contrast to the above relationship when the loss of chlorohyll is due to nutritional causes or to aging of the plant, the chlorophyllase decreases. Whether or not this relationship maintains in plants other than tobacco, or in plants with other chloroses, has not been ascertained. It is

possible that the chlorophyllase level might be used as a quick method for distinguishing certain types of chlorosis.

SUMMARY

A method of determining the chlorophyllase activities of plant tissues based on procedure established by Willstätter is described.

The "green weight" of tobacco leaves was found to be comparable to area as a basis for making plastid-pigment and chlorophyllase comparisons. Dry weight was less comparable to area because of the comparatively low dry weight of yellow mosaic plants. The chlorophyll content of this type, when corrected for low dry weight, was out of line with visible evidence of chlorosis.

The chlorophyll content of tobacco leaves affected with common mosaic, yellow mosaic, mild dark-green mosaic, and the mild mosaic was found to be consistently lower than that of healthy plants.

The drop in chlorophyll content caused by mosaic was found to be associated with an approximately proportional drop in the yellow pigments, carotene, and xanthophyll.

With the exception of common mosaic, the chlorophyllase activity of the ground leaf tissues of mosaic plants averaged appreciably higher than that of healthy tobacco.

A reduction of 50 per cent in the chlorophyll content of yellow mosaic plants, compared with healthy tobacco, is associated with an approximate doubling of the chlorophyllase activity of the ground leaf tissues. The yellow areas were found to be lower in chlorophyll but higher in chlorophyllase activity than the green areas.

In contrast to the above relationship, the chlorophyllase activity of healthy leaf tissues was found to be directly proportional to chlorophyll content. A 50 per cent reduction in the chlorophyll content, middle leaves compared with top leaves, was found to be accompanied by a similar reduction in chlorophyllase activity.

In a comparison of leaf, stem, and root tissues of yellow mosaic plants, the chlorophyllase activity was found to be proportional to chlorophyll content. Chlorophyllase activity was very low in pith and root tissues that were devoid of chlorophyll.

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THE LONGEVITY OF CERCOSPORA BETICOLA IN SOIL[1]

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The source of initial infection of *Cercospora beticola* Sacc., on sugar beets under field conditions is not thoroughly understood. Vestal (6) reported that certain weed hosts may initiate primary leaf-spot infection, while Pool and McKay (4) reported that primary infection was due to the overwintering of the organism on old beet tops. Vestal also suggested that *C. beticola* not only lived but also reproduced saprophytically on dead tissues of the sugar beet and several weed hosts. Massee (1), Pammel (3), and Thümen (5) believed that the spores were able to live for a time in the soil and retain their pathogenicity. The writer, in a few cases, has recovered the organism from old beet refuse.

Continuous cropping to sugar beets markedly increases the prevalence of *Cercospora beticola.* A four-year rotation system reduces the damage caused by this pathogen. Primary and secondary infections usually are sufficiently delayed where a crop-rotation system is followed, so that the accumulative increase of the pathogen during the season, even under favorable environmental conditions, seldom reaches the epiphytotic stage.

Pool and McKay (4) reported that the conidia, when exposed to outdoor conditions, died in 1 to 4 months, but, when kept dry, might live 8 months. The sclerotia-like bodies imbedded in the host tissue lived through the winter when protected in the center of a pile of beet tops or when buried in the soil 1 to 5 inches. Pool and McKay also believed that the organism under the above-named conditions becomes a source of primary infection the following year.

The writer in an abstract (2) reported that *Cercospora beticola* grew well and retained its pathogenicity in 5 different kinds of sterile soil for 27

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months, and that abundant leaf-spot infection developed on cotyledons and leaves of sugar-beet seedlings grown on naturally infested field soil in the greenhouse.

GROWTH OF CERCOSPORA BETICOLA IN STERILE SOIL CULTURES

Cercospora beticola was grown on 5 different soils obtained in the vicinity of Kanawha, Iowa. These soils consisted of peat, basic black loam, acid black loam, black loam low in organic matter, and neutral black loam. The pH varied from 6.0 to 7.4, as shown in table 1. The soil was uniformly

TABLE 1.—*Growth of Cercospora beticola on five different sterilized soils collected at Kanawha, Iowa*

Soils tested	pH	Mycelial growth (av. diam. in cm.)
Peat	6.4	6.4
Basic black loam	7.4	3.8
Acid black loam	6.0	3.6
Black loam low in organic matter	6.9	3.1
Neutral black loam	6.8	5.0

moistened by the addition of water until a friable state was obtained, then placed in Petri dishes and firmly packed, leaving about a 3 mm. space between the level of the soil and the top of the Petri-dish cover. Thirty dishes, 6 replications of the 5 soils, were steam-sterilized and then uniformly inoculated by placing an aqueous conidial suspension of the organism on the surface of the soil in the center of each dish. The sterile, inoculated-soil cultures were placed in a moist chamber to prevent evaporation and incubated at 25° C. The rate of growth of the fungus (Table 1) was calculated by measuring the diameter of the surface growth of the mycelia during ten 24-hour intervals. No quantitative growth measurements were taken, but it was noted that the mycelia uniformly penetrated the soil.

During the early development of the cultures, conidial production was observed in all cases. The best vegetative growth occurred on the peat and neutral black loam. There apparently was no correlation between pH and rate of growth, but the soils that were the highest in organic matter produced the most vegetative growth.

LONGEVITY OF CERCOSPORA BETICOLA ON STERILIZED SOIL CULTURES

Portions of the 5 different soils listed in table 1 were moistened with distilled water to a uniform friable state, and each was used to half fill six 250 cc. Erlenmeyer flasks. After sterilization the soil portions were uniformly inoculated. The cultures were then placed in a large moist chamber for 59 days at a temperature of 24°–26° C. During this time the organism had spread generally through the soil. The cultures were then removed and exposed to laboratory temperature where they soon attained air-dry conditions. From each set of the 5 different types of soil, 1 culture of each was placed outdoors throughout the winter months. During this period the

temperature varied from $+15.1°$ to $-27.8°$ C. A duplicate set, and also a third set consisting of 4 flask cultures of each kind of soil were held under laboratory conditions.

The 2 sets of cultures, 1 of which was kept outdoors, the other in the laboratory, were used to inoculate young sugar beet plants in the greenhouse. At intervals of 18, 27, and 40 months, inoculations of sugar beets were made (Table 2). The method of growing the beet plants, and of preparation of

TABLE 2.—*Period during which Cercospora beticola remained pathogenic in sterilized soil cultures*

Series	Soil culture		Number of plants	
	Number	Age	Inoculated	Diseased
I	1 ck.	18 mos.	45	0
	2[a]	"	57	28
	3	"	60	35
	4[a]	"	56	1
	5	"	54	30
	6[a]	"	36	0
	7	"	55	18
	8[a]	"	47	28
	9	"	77	38
	10[a]	"	46	35
	11	"	50	25
II	1 ck.	27 mos.	70	0
	2[a]	"	50	17
	3	"	47	16
	4[a]	"	58	0
	5	"	60	10
	6[a]	"	40	0
	7	"	62	18
	8[a]	"	48	23
	9	"	70	32
	10[a]	"	65	36
	11	"	39	0
III	1 ck.	40 mos.	40	0
	2[a]	"	43	0
	3	"	36	0
	4[a]	"	32	0
	5	"	39	0
	6[a]	"	38	0
	7	"	48	0
	8[a]	"	45	0
	9	"	39	0
	10[a]	"	31	0
	11	"	42	0

[a] Cultures exposed for 3 months to winter weather conditions.

the inoculum was as follows: beet seed, treated with New Improved Ceresan, was planted in pots of steamed soil. When the plants had reached the 3-leaf stage, they were placed in a moist chamber and sprayed with water, so that dry powdered soil would adhere to the leaves. The plants were then exposed to infection by sprinkling the finely powdered soil culture over the leaves with the aid of a screen. Thirty-six hours later the plants were

removed to the greenhouse bench for the remainder of the incubation period. Leaf-spot readings were taken 12 days after inoculation (Table 2, Series I).

At the end of 27 and 40 months, the same procedure was followed, using the same cultures as in series I. The results (Series II) show that there was a reduction in the amount of pathogenic-fungus material in the 27-month-old cultures as compared with the results obtained in series I, in which the cultures were 18 months old.

At the end of approximately 40 months, the same cultures were again tested for viability. The results presented in series III show that after 40 months apparently no pathogenic-fungus material was present in any of the cultures at the time of inoculation.

LONGEVITY OF CERCOSPORA BETICOLA IN NONSTERILIZED FIELD SOIL

Isolation trials to obtain the pathogen from naturally infested soils by the plate-dilution method were failures, probably because the pathogen is a comparatively slow grower. The method used in the inoculation experiments, namely, sprinkling dry soil on the moistened beet leaves, was successful in isolating the pathogen from naturally infested soil, but a small percentage of the beet plants were killed by sprinkling the nonsterilized soil on the young sugar-beet seedlings.

Later, it was found that by planting the treated sugar-beet seed directly in flats of Cercospora-infested soil, good results could be obtained. The earliest infection occurred on the cotyledons and later on the leaves. The inoculum in the soil was brought in contact with the cotyledons and leaves when the plants were watered, and also when the plants wilted, permitting the leaves to lie on the ground until favorable environmental conditions restored them to normal. The beet leaves apparently can be in a wilted state for many hours without visible injury. Wilting of the leaves frequently occurs under field conditions during unusually hot days. From field and greenhouse observations it has been observed that the beet leaf in this condition collects considerable moisture on the lower surface next to the soil, and this acts as a moist chamber and thereby permits infection, if the organism be present. Initial leaf-spot infection may develop in this manner under what might be assumed to be apparently unfavorable conditions for growth of the organism in the field. A general primary infection thus induced and followed by high humidity or rain usually develops rapidly into a severe leaf-spot epiphytotic over the entire field. The occurrence of leaf-spot epiphytotics, such as described above, are frequently observed under field conditions in Iowa.

In order to study this phase of the problem under controlled conditions, soil was collected in the fall of 1934 from a Kanawha field that had grown sugar beets the previous season. In collecting this soil, the upper 1 inch was removed and discarded. The next layer of 3 to 4 inches was placed in containers and taken to the greenhouse at Ames. Fragments of plant débris were removed. Other soils tested consisted of greenhouse compost, to which

was added an aqueous conidial suspension of *Cercospora beticola.* Still another series consisted of compost without the addition of the organism. Naturally infested steamed field soil was used as the check. The results of this experiment are presented in table 3, series I, II and III. The experiment extended over a period of 20 months. During this time 3 different disease readings were made, the same soil being left in its respective flat, and each of the 3 plantings were made in the same soil.

Treated seed was used to plant 3 rows in each of 3 flats in each series After each leaf-spot reading, the plants were carefully pulled from the soil so as to leave no host tissue, particularly leaf tissue. The first seedlings in series I emerged January 1, and the leaf-spot reading on cotyledons and leaves was made on January 30 (Table 3, Series I). The 12 flats were then carefully wrapped individually in stiff brown wrapping paper and placed outdoors during the balance of the winter months. On May 1, these flats were returned to the greenhouse, unwrapped, the soil allowed to thaw out, and again planted to sugar beets, in the manner described. On June 17, the data were taken on series II. The results, when compared with those of series I, indicated that there had been some decrease in the amount of *Cercospora* present in the soil. Approximately 6 months later no infection, for some unknown reason, had resulted in the case of the compost soil.

After the second count was taken, the plants were again removed, as previously mentioned in the case of series I. The flats were left on a greenhouse bench for 1 month, then removed to the cold room at 5° C. for 3 months, then placed in a second room at a temperature of $-7°$ C. for 6 months. Immediately thereafter the flats were returned to the greenhouse and left for 2 days, or until the soil had completely thawed and attained the greenhouse temperature. Sugar-beet seed was again sown and, on May 30, the data in series III were completed. The results in table 3, series III, show a marked decline in the population of the Cercospora-infested field soil.

The viable period of this pathogen in sterile soil appears to be rather closely correlated with that period during which the organism can remain in nonsterile soil and still be pathogenic to its host, under the conditions of these experiments.

FIELD INFECTIONS SUGGESTING THE SOIL AS THE SOURCE OF INOCULUM

Dates of the earliest observed primary leaf-spot infection in Iowa over the past several years have been variable. This variability is no doubt influenced by such factors as source of infection, environmental conditions, and crop rotation. In Iowa it has been observed that sugar-beet leaves become infected with *Cercospora beticola,* provided they come in direct contact with the soil. This may be brought about in the seedling stage particularly, just after singling (3- to 4-leaf stage). The disturbance to the plants during the process of singling causes them to lose their turgor and fall to the ground, where they may remain for several days. In this condition, the wilted leaves on the ground collect abundant moisture on the lower surface

TABLE 3.—*Period during which Cercospora beticola remained pathogenic in Cercospora-infested field soil*

Series	Soils and treatments	Age soil from date collection	Plants	Diseased plants	Cotyledon inf.	Leaf inf.	Cot. and leaf inf.	Reisolations
		Months	*Total No.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
I	*Cercospora*-infested field soil	4	338	71.9	68.9	27.2	23.9	100
	Cercospora-infested field soil, steamed	4	506	0	0	0	0	0
	Compost, plus conidial suspension	4	570	9.2	6.8	1.5	1.5	100
	Compost	4	500	0	0	0	0	0
II	*Cercospora*-infested field soil	9	474	66.7	4.2	37.6	24.9	90
	Cercospora-infested field soil, steamed	9	529	0	0	0	0	0
	Compost, plus conidial suspension	9	416	0	0	0	0	0
	Compost	9	472	0	0	0	0	0
III	*Cercospora*-infested field soil	20	265	5.3	1.6	4.5	1.5	100
	Cercospora-infested field soil	20	301	0	0	0	0	0
	Compost, plus conidial suspension	20	252	0	0	0	0	0
	Compost	20	278	0	0	0	0	0

and in this way set up ideal conditions for infection. Heavy rains may induce a similar condition in the case of young beet plants.

In cases where infection takes place later in the season when the foliage of the plants is mature, it is the outer leaves that become infected first. Frequent observations are necessary in order to observe this condition and to note the relative position of those leaves that bear the initial symptoms. Later, the inoculum is passed to inner leaves and epiphytotic conditions rapidly develop.

On land where beets follow beets and where leaf spot occurs each year, the data indicate that there is built up in the soil an increasing amount of the leaf-spot organism. Primary infection occurs from 2 to 3 weeks earlier on land where beets follow beets and develops into epiphytotic proportions more rapidly under normal weather conditions in Iowa.

In 1932 a 2-acre field of sugar beets developed a severe epiphytotic of Cercospora leaf spot, which defoliated the plants. In 1933 the same field, and also an additional acre immediately bordering it, was sown to beets on April 27; and in this case the entire 3 acres were treated as a unit. Under these circumstances adequate opportunity for dissemination of the inoculum was afforded by the distribution of beet refuse by wind, rain, and cultivation during the usual operations incident to sowing and later to the cultivation of the crop.

In midseason of 1933 a severe epiphytotic of leaf spot occurred on that portion of the field cropped to beets the second season. The severe leaf-spot damage was limited to the original 2-acre area. Approximately 2 crops of leaves were defoliated prior to harvest. The bordering additional acre that had not grown beets the previous season showed only light infection, involving little defoliation.

The yields and sugar percentages taken at harvest indicated the relative severity of leaf spot on the 2 portions of the field. The average sugar percentages and acre yields were as follows: beets following beets: percentage sucrose 9.6 per cent, yield 13.9 tons; first year beets: percentage sucrose 12.2 per cent, yield 23.9 tons. In 1933, there was no way of estimating the fertility differences between these two plots. However, there was no significant difference in yields of corn grown on these same plots during the 1934 season, which was also true when barley was grown on these same plots in 1935. Therefore, it seems reasonable to ascribe the large difference shown above to the differences in severity of leaf-spot infection.

In 1933, the first leaf-spot symptoms appeared in the narrowest spacings of the experimental sugar beet plots. The plants were in about the 6- to 8-leaf stage. Only the lower leaves showed a light amount of spotting. These observations were made just after singling. No additional symptoms appeared until the plants had produced approximately a full set of leaves. At this stage of development, the outer leaves usually come in contact with the soil, and it is largely these leaves that obtain the primary infection from the soil. Later, under favorable conditions, secondary infection spreads to the inner or upper leaves.

Again, in 1933, sugar beets were planted on a 20-acre field that had not been cropped to beets for several years. Detailed data were taken in the field on the presence and amount of leaf spot on 23 one-square-rod plots taken at random over the entire field. On August 25, of the 1682 plants examined, 23.18 per cent were healthy and 76.82 per cent had a light infection, consisting of from 1 to 10 spots per plant. In nearly all cases the spots occurred on the lower or outer leaves. No infection "centers" were present in the fields that might have been responsible for the dissemination of inoculum.

In 1934 the first leaf-spot occurrence was on July 25. The plants were in about the fourth leaf stage, due to late planting. The general but light infection on the lower leaves occurred just after singling, succeeded by heavy rains.

Data obtained in 1935 in which several thousand individual leaf counts were made on approximately 1000 plants in the initial stages of leaf-spot development, showed that the symptoms were present almost entirely on the lower leaves.

SUMMARY

This paper presents evidence showing that *Cercospora beticola* can live in the soil, and that it spreads from the soil to the cotyledons and leaves of the sugar-beet plant.

Cercospora beticola grew well on at least 5 different kinds of soil. At the end of 18 and 27 months, respectively, the organism retained its viability and pathogenicity in sterile soil cultures, while at the end of 9 and 20 months, respectively, using naturally infested field soil, the pathogen retained its virility, but there was a marked decline in the amount of *Cercospora* remaining in the soil at the end of 20 months, as determined by the amount of initial infection.

Abundant conidial production occurred in young soil cultures in all cases.

It was found also that this fungus will tolerate a temperature range of a + 15.5° to a − 27.8° C. in sterile soil cultures without losing its viability or pathogenicity.

A method is described for isolation of the pathogen from the soil indirectly by means of the host plant.

There apparently is no relationship between pH and the amount of mycelial development at a range of 6.0 to 7.4 in the case of the soils tested in this experiment. However, there was a more rapid growth of the mycelium on the soils containing an abundance of organic matter.

When sugar beets were grown a second year on the same field severely infested with *Cercospora beticola* the preceeding year, leaf spot was very destructive and the yield and percentage of sucrose were markedly depressed. Relatively little infection with a higher yield and percentage of sucrose resulted on adjacent land that had not been cropped to beets for several seasons.

BOTANY AND PLANT PATHOLOGY SECTION
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A SOFT-ROT BACTERIOSIS OF PUMPKIN FRUITS[1]

P. A. ARK AND C. M. TOMPKINS

(Accepted for publication January 20, 1938)

An epidemic of soft rot of pumpkin fruits was observed by the authors in September, 1935, near San Pablo, California. In a preliminary report,[2] the cause was ascribed to a bacterium closely related to *Erwinia carotovora*. Since then, the disease has not been observed in epidemic form, although mild cases occurred in various coastal areas in the State in 1936 and 1937. The purpose of this paper is to discuss more fully the causal agent and its host range.

The disease usually affects only young pumpkin fruits. The affected area, which at first appears water-soaked, soon changes into a soft mass, lacking in mechanical firmness, and possessing a disagreeable odor. Frequently, species of *Fusarium* and *Botrytis*, as well as *Rhizopus nigricans* Ehrenberg, are also found on fruits affected by the bacterial soft rot. A form of *Fusarium javanicum* Koord (kindly identified by W. C. Snyder), often found in association with the soft-rot bacteria, is capable of invading detached fruit in moist chambers without wounding (Fig. 1), but in association with bacteria the damage is greater (Fig. 1). The disease does not affect the leaves, stems, or roots of the pumpkin plant.

Plants Affected.[3] Varieties susceptible to the bacterial soft rot (field observations and greenhouse tests) are as follows: *Cucurbita maxima* Duchesne (Squash—var. Aizu Kuzi, Banana, Boston Marrow, Golden Delicious, Green Delicious, Hubbard Blue, Hubbard Golden, Hubbard Kitchenette, Hubbard Warted, Mammoth Chili, Mammoth Whale, Warren); *C. moschata* Duchesne (Cushaw—var. Aizu Wase (Japan), Australian Bush, Green Striped, Japanese Pie, Kentucky Field, Large Cheese, Shishigatani (Japan)); *C. pepo* L. var. *condensa* Bailey (Pumpkin—var. Boston Creek,

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California. The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

[2] Ark, P. A., and C. M. Tompkins. Bacteriosis of pumpkin fruits in California. Science (n. s.) 84: 18. 1936.

[3] The writers are indebted to Mr. B. F. Dana, U. S. Dept. of Agriculture, Corvallis, Oregon, for kindly supplying fruits of numerous pumpkin varieties for inoculation purposes.

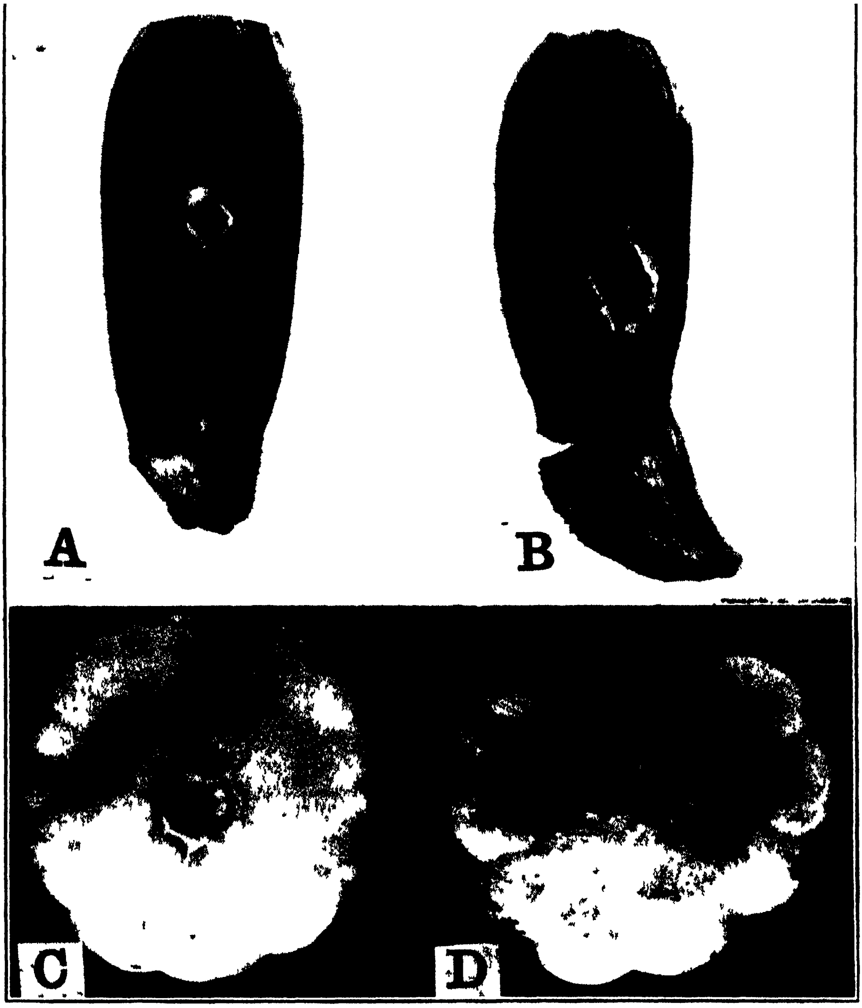

FIG. 1. A and B. *Cucurbita pepo* L. var. *condensa* Bailey (var. Zucchini). C and D. *C pepo* L. var. *condensa* Bailey (var. Early White Bush Scallop). Blocks of agar with mycelium of the form *Fusarium javanicum* Kooid. (A and C). The same fungus as in A and C plus the bacterial organism from pumpkin (B and D). Four days after inoculation. No wound was made in either case.

Cocozelle, Danish, Delicata or Sweet Potato, Early Summer Crookneck Yellow, Early Prolific White Bush, Early White Bush Scallop, Fordhook Bush, Giant Summer Crookneck, Orange Winter Luxury, Pie, Table Queen, Vegetable Marrow Green Vining, Vegetable Marrow Long White Bush, Yellow Crookneck and Zucchini); *C. pepo* var. *ovifera* Bailey (Gourd—var. *maliformis* (Lithuania), *pyriformis verrucosa* (Lithuania), *striata elongata* (Lithuania)); *Lagineria leucantha* Rusby (White-flowered gourd). Early White Bush Scallop (Figs. 2 and 3), Yellow Crookneck, and Zucchini pumpkin fruits were found to be especially susceptible to the bacterial rot under field conditions. Resistance was found only in *C. moschata*, in an unknown vari-

FIG. 2. Natural infection of pumpkin fruits (*Cucurbita pepo* L. var. *condensa* Bailey) var. Early White Bush Scallop.

ety from India, and in gourds (var. *pyriformis striata* and *maliformis latea*) from Lithuania.

The soft-rot organism from pumpkin is also pathogenic to other species of plants when introduced into plant parts by a needle contaminated with the organism. Table 1 shows results of inoculating various plants. Because celery plants (*Apium graveolens* L.) are sometimes severely damaged in the field by the organism (soft rot of crown and roots), numerous varieties of celery were tested in the greenhouse (Fig. 4). Large plants in 6-inch pots were inoculated in the crown and placed outdoors. The following varieties of celery were found to be susceptible: Florida Golden, French Long Top, Golden Phenomenal, Golden Plume, Golden Plumé Hybrid, Lagomarsiño Special, Long Standing Golden Plume, Tall Paris Golden Yellow, Tethers Special French Tall Strain, and Yellow Hybrid, while Golden Detroit, Utah or Golden Crisp, and Wild Type No. 2 were resistant to the soft-rot bacteria. It is of interest to note that, although several strains of *Erwinia carotovora* from this laboratory failed to cause any effect on *Opuntia* spp., the pumpkin

FIG. 3. Pumpkin fruits naturally infected by the pumpkin soft rot organism. A. Zucchini. B. Danish C. Early White Bush Scallop.

soft-rot organism disintegrated fleshy parts of some representatives of that genus.

Causal Agent. In the preliminary report by the authors,[4] it was stated that the organism appeared to be related in many respects to *E. carotovora* (L. R. Jones) Holland. Subsequently, more extensive comparative studies were made of cultural and physiological characters of the organism and it has been identified as *E. aroideae* (Townsend) Holland. There were some small differences between certain isolates and the authentic *E. aroideae.* These differences, however, were not great enough, in the opinion of the writers, to preclude the designation of the pumpkin organism as *E. aroideae. E. aroideae* from pumpkin appeared to attack hosts that were not attacked by the authentic culture.

[4] See footnote 2.

TALBE 1.—*Inoculations of the soft rot organism of squash fruit into different plants*

Plant	Part inoculated	Result	Plant	Part inoculated	Result
Allium cepa L.	Bulb	+	*Lycopersicum esculentum* Mill. var. *vulgaris* Bailey	Fruit	+
Ananas sativus Schult	Fruit	+	*Matthiola incana* R. Br. var. *annua* Voss.	Seedlings	+
Apium graveolens L.	Crown[a]	+	*Phaseolus limensis* M.	"	+
Beta vulgaris L.	Root	−	*Ph. vulgaris* L.	Pod	−
B. vulgaris L. var. *crassa* Alef.	"	−	*Pelargonium hortorum* Bailey	Cuttings	−
Brassica oleracea L. var. *botrytis* L.	Head	+	*Vicia faba* L.	Seedlings[b]	+
B. oleracea L. var. *capitata* L.	"	+	*Opuntia basilaris* Eng. & B.	Leaf	−
Capsicum annuum L. var. *grossum* Sendt.	Fruit	+	*O. basilaris* Eng. & B. var. *treleasii* Coult.	"	−
Citrus limonia Osbeck	"	−	*O. haematocarpa* Berger	"	−
C. sinensis Osbeck	"	−	*O. inermis* De Candolle	"	−
Citrullus vulgaris Schrad.	"	+	*O. linguiformis* Griffiths	"	−
Cucumis melo L.—var. US45, US619 & US620	"	+	*O. macrocentra* Eng.	"	+
C. melo var. *cantalupensis* Naud.	"	−	*O. mieckleyi* Schumann	"	+
C. melo var. *inodorus* Naud.	"	−	*O. monocantha* Haw.	"	−
C. melo var. *reticulatus* Naud.	"	+	*O. robusta* Wendland	"	+
C. sativus L.	"	+	*O. septacantha* Haw.	"	+
Daucus carota L. var. *sativa* DC.	Root	+	*O. senilis* Parmenteer	"	+
Delphinium ajacis L.	Seedlings	−	*O. vulgaris* Mill.	"	
Lactuca sativa L.	Head	+	*O. velutina* Weber	"	−

[a] Also petiole.
[b] Also pods.

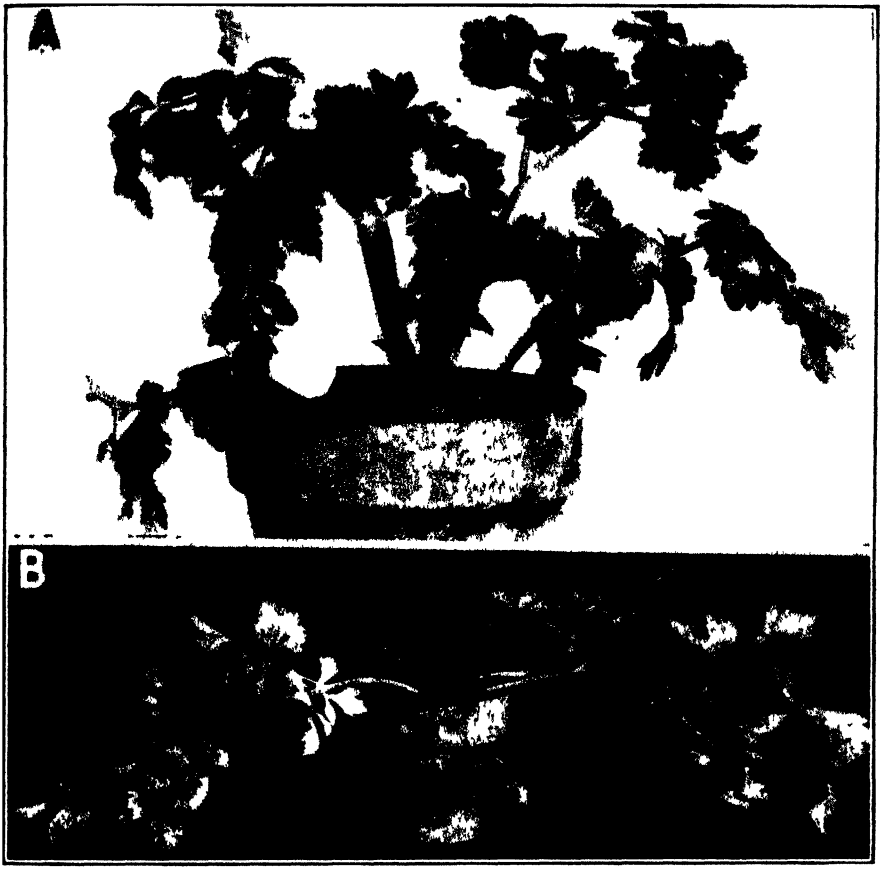

FIG. 4. A. Celery plant sprayed with the culture of the pumpkin soft-rot organism. Four days after spraying. B. Celery plant inoculated in the crown with an agar-slant growth of the pumpkin soft rot organism. Two weeks after inoculation.

SUMMARY

The bacterial soft-rot organism of pumpkin in California attacks a large number of pumpkin, celery, and also other varieties of plants under natural conditions.

Several resistant varieties of pumpkin and celery are reported.

The causal organism was identified as *Erwinia aroideae* (Townsend) Holland.

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SPOROPHORE FORMATION BY FOMES APPLANATUS IN CULTURE[1]

HENRY HOPP[2]

(Accepted for publication January 4, 1938)

The formation of typical sporophores by species of *Fomes* growing in culture is not a common occurrence. White[3] obtained fruits of *Fomes applanatus* (Pers.) Wallr. in culture, but they were abnormal in shape and appearance. The failure of mycelium to form normal fruiting bodies in culture was attributed to an insufficient supply of food provided by the small pieces of wood used as a substratum. In the present study, however, it has been found possible to induce the formation of typical fertile sporophores of *F. applanatus* by cultural methods. The strain of *F. applanatus* used in these tests was isolated from the context of a sporophore attached to a fallen log of *Fagus grandifolia* Ehrh. and has been grown since on agar substrata. During this period of over 4 years, the isolate has neither fruited nor shown any significant difference from isolations of this species obtained from several other sources and grown in stock culture under similar conditions.

Cubical wood blocks of *Populus canadensis* var. *eugenie* Schelle., which measured 5 cm. on a side, were used in the present study as a substratum for the fungus. They were partially evacuated of air by soaking them alternately in boiling and cold water until they sank. Each block was then placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker, and water was added up to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton, which was held in place by the inverted half of a Petri dish. The culture blocks were then sterilized at 17 lbs.' pressure for 30 minutes. After sterilization, small pieces of mycelial tissue taken from a stock culture were placed on the blocks. The cultures on the wood blocks were then incubated at 28° C. and 100 per cent relative humidity.

After 5 to 6 weeks, when the mycelium was well established in the wood, half the number of blocks were transferred under aseptic conditions to sterilized humidity chambers, the construction of which has been previously described (pp. 19 and 30).[4] The blocks were subjected in these chambers to controlled conditions of aeration and humidity. The chambers were exposed to the diffused daylight of the laboratory. The blocks were so placed inside the chambers that the grain of the wood was oriented in the vertical direction.

Three wood blocks were placed in each of 5 humidity chambers, which

[1] This study was performed while the writer was a graduate assistant in the Department of Forest Botany and Pathology, New York State College of Forestry, Syracuse, New York.

[2] Associate Botanist, Section of Hill Culture Research, Soil Conservation Service, U. S. Department of Agriculture.

[3] White, J. H. On the biology of *Fomes applanatus* (Pers.) Wallr. Roy. Canad. Inst. Trans. 12: 133–174. 1920.

[4] Hopp, H. Control of atmospheric humidity in culture studies. Bot. Gaz. 98: 25–44. 1936.

were subjected to different percentages of relative humidity by use of the substances mentioned in table 1. In addition to the 15 blocks placed in these aerated chambers, an equal number of decaying blocks were left in the original culture beakers, and were, therefore, incubated in nonaerated vessels. The experiment was subsequently repeated, so that a total of 60 blocks were used in the course of the entire study, 30 of which were exposed to aeration and controlled humidity; and 30 to nonaeration and 100 per cent relative humidity. The results of the 2 trials were similar.

In the course of 1 to 3 weeks, 14 fertile sporophores developed on 11 of the 30 blocks placed in the aerated humidity chambers. Most of the sporophores were located on the vertical sides of the blocks, but some were on the top surface. In most cases, the sporophores were atypical, in that either a

FIG. 1. Sporophore grown on a wood block of *Populus canadensis* var. *eugenie* that had been subjected to decay by *Fomes applanatus* in culture. The block was exposed to a continuous current of air at 75 per cent relative humidity. × 2.

normal pileus was lacking or the tubes were not oriented in a vertical direction. These atypical forms appeared similar to those described by White. No sporophores appeared during the same period of time on the corresponding 30 blocks incubated in nonaerated vessels.

Four of the 14 sporophores were considered to be normal in appearance and structure. The 4 typical fruiting bodies formed on the vertical sides of those blocks that were subjected to 75 per cent relative humidity (Table 1).

TABLE 1.—*Sporophore production of Fomes applanatus grown on wood blocks in aerated humidity chambers*

Percentage relative humidity	Substance used to control humidity	Total No. of blocks	No. of blocks bearing sporophores	No. of fertile sporophores	No. of typical sporophores
0	Anhydrone	6	0	0	0
35	$MgCl_2 \cdot 6H_2O$	6	0	0	0
53	$CaNO_3 \cdot 4H_2O$	6	3	4	0
75	NaCl	6	5	6	4
100	Distilled H_2O	6	3	4	0

They eventually attained a length of 2 to 3 cm. One of the sporophores is illustrated in figure 1.

As far as known, this is the first time that the formation of typical shelving sporophores of a *Fomes* was induced by cultural methods. In this study, typical sporophores appeared when the cultures were aerated at 75 per cent relative humidity. The environmental conditions conducive to the formation of normal sporophores by *F. applanatus*, therefore, may be defined as follows: (*a*) Exposure of the surface mycelium to ventilation with air of normal oxygen concentration; (*b*) sufficient moisture supply to the mycelium within the substratum; (*c*) continuous but moderate desiccation of the surface mycelium by exposure of the wood block to moist but not saturated air. Although the physiological rôle of these factors was not certain, it appeared that initiation and formation of the sporophores were related to ventilation of the mycelium with "fresh" air (probably oxygen of normal concentration); and that the typical orientation and coloration of the parts of the sporophores were conditioned by the atmospheric humidity.

PHYTOPHTHORA CACTORUM ASSOCIATED WITH SEEDLING DISEASES IN FOREST NURSERIES

BOWEN S. CRANDALL AND CARL HARTLEY

(Accepted for publication January 27, 1938)

During routine isolation work on forest-tree seedlings affected with root rots and damping off of the sore-shin and top-wilt type, *Phytophthora cactorum*, occasionally, has been isolated. Observations on its presence in forest-tree nurseries in this country are of some interest because pathologists in Europe have sometimes reported it, under the synonym *P. fagi* or *P. omnivora*, as causing heavy losses on a great variety of coniferous and deciduous hosts.

In this country Pierce's *Phytophthora* sp. from *Pinus resinosa*, later described by Leonian[1] as *P. pini* n. sp., but closely resembling or identical with *P. cactorum*, has been reported as causing damping off of pine.[2] Inoculation experiments have indicated that it is a weak damping-off pathogen on *Pinus banksiana*,[3] *Pinus ponderosa*,[2] and *Pinus resinosa*.[3] Additional published reports, which indicate that *P. cactorum* is a common cause of dieback and cankers on a number of broadleaf tree species, have been made by various workers as a result of isolations from naturally infected material or on the basis of artificial inoculations.

[1] Leonian, L. H. Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. 12: 444–498. 1925.

[2] Hartley, C. Damping-off in forest nurseries. U. S. Dept. Agr. Bull. 934. 1921.

[3] Gravatt, Annie Rathbun-. Direct inoculation of coniferous stems with damping-off fungi. Jour. Agr. Res. [U. S.] 30: 327–339. 1925.

——————. Germination loss of coniferous seeds due to parasites. Jour. Agr. Res. [U. S.] 42: 71–92. 1931.

See also footnote 2.

According to unpublished reports to the writers, *Phytophthora cactorum* also has been isolated at an Ohio nursery from seedlings of European beech showing symptoms of top wilt[4] and from tulip poplar seedlings with typical sore shin.[5] Some recent findings by the writers are reported in the following paragraphs:

Shortly after emergence *Juglans nigra* seedlings in a North Carolina nursery, both in beds sown broadcast and in rows, suddenly wilted and died. The wilted seedlings were found to have root infections of the soft-rot type. Examination of the blank spaces in beds and rows showed that approximately 50 per cent of the seedlings or seed had been lost before emergence. The infections apparently had started as soon as, or before, the nuts had germinated and had continued as a root rot. In some portions of beds and rows a total loss eventually occurred. Plantings in agar of tissue from the margins of lesions on infected seedlings yielded pure cultures of *Phytophthora cactorum*. Greenhouse inoculations were made on 5-year-old trees by inserting rice, on which one of the isolates was growing, in a cut made at the collar. After inoculation the cut, which extended through the bark to the cambium, was covered with dry sterile cotton and linen tape. Root rot with symptoms similar to those found in the field occurred on 3 of the 5 inoculated trees. No rot resulted from check inoculations made with sterile rice on an equal number of trees.

Seedlings of *Nyssa sylvatica*, *Colutea arborescens* and *Caragana arborescens* at an Elsberry, Missouri, nursery suffered severe losses from infections that appeared to have originated above ground. The injury was characterized by softening of the leaves and upper stem; in the seedlings not too far gone at the time the nursery was visited, the roots were still normal in appearance. *P. cactorum* was isolated from the seedlings of all 3 species after shipment to Washington.[6] Estimated losses were as follows: *Nyssa* 80 per cent, *Colutea* 25 per cent, and *Caragana* 100 per cent. Similar losses occurred at the same time, in the same area, and presumably from the same cause, on species of *Cornus, Robinia, Acer, Prunus,* and *Ostrya*. However, the material of these species reached the laboratory in poor condition and yielded no recognized parasite.

An 80 per cent loss of 4-week-old *Pinus nigra* seedlings sown broadcast in beds was experienced at a single Maryland nursery. The disease was characterized by softening and discoloration of the collar and portions of the root. *Phytophthora cactorum* was isolated from portions of infected seedlings planted in agar.

The strain from *Pinus resinosa* came from a very sandy soil; most of the writers' isolations were from seedlings growing on heavy soils in cool and abnormally wet weather.

[4] Note from Curtis May, Dutch Elm Disease Laboratory, Morristown, New Jersey.

[5] Oral report from Roger Swingle, Dutch Elm Disease Laboratory, Wooster, Ohio.

[6] The identification of the fungus was verified by C. M. Tucker and the occurrence reported in W. E. Maneval's "A list of Missouri Fungi . . ." published in Missouri Univ. Studies, v. 12, no. 3. 1937.

No seedlings of *Nyssa, Colutea, Caragana,* or *Pinus nigra* were available for inoculation. To test the degree of similarity between the strains from them and the so-called *Phytopthora pini,* inoculations were made with isolates of *P. cactorum* from the 4 above-mentioned hosts in comparison with Pierce's original isolate of *P. pini* from *Pinus resinosa* in Minnesota. Ten 3-year-old seedlings were inoculated in a wound at the collar with isolates from each of the species mentioned, and 16 with *P. pini.* *P. pini* killed 15 out of the 16 seedlings inoculated with it; all inoculations with isolates from the other hosts were negative. Check inoculations were also negative.

DIVISION OF FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
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ADDITIONAL RECORDS OF ROSE ANTHRACNOSE IN THE UNITED STATES

ANNA E. JENKINS AND FRANK P. MCWHORTER

(Accepted for publication February 21, 1938)

Since the identification of rose anthracnose (*Sphaceloma rosarum* (Pass.) Jenkins)[1] in the United States, certain new and more or less historical records of the disease have been obtained and have been employed in a map[2] showing the localities in the United States whence the disease has definitely been reported. The sources of these additional records are given here, except that for Kansas, which is reported elsewhere.[3]

NORTH CAROLINA

When the distribution of rose anthracnose was given in 1932,[4] the only available records for North Carolina were 2 phanerogamic specimens of the commercial rose varieties Alex Tremouillet and Valentine Beaulieu from Biltmore, N. C., collected in June, 1909, by P. L. Ricker.[5] Additional early records of the disease in this State have been obtained through the recent accession of several previously unidentified mycological specimens of rose fungi from North Carolina that proved to be of *Sphaceloma rosarum.* Contributed from the herbarium of the late F. L. Stevens, these are cited as follows: (Locality not given), June 21, 1902, and June 15, 1904, F. L. Stevens; West Raleigh, Sept., 1903 and 1907, F. L. Stevens, and Sept. 21, 1905, S. W. Foster; Bakersville, July 8, 1905; Huntersville, July 27, 1907,

[1] Jenkins, A. E. Rose anthracnose caused by Sphaceloma. Jour. Agr. Res. 45: 321–337. 1932.

[2] Jenkins, A. E., and L. M. Massey. Rose anthracnose. American Rose Annual 1938: 136–141. 1938.

[3] Dearness, J. *Sphaceloma rosarum* (Pass.) Jenkins, as *Gloeosporium rosaecola* Dearn. and Barth. nomen nudum. Mycologia 29: 1938. (In press.)

[4] *Loc. cit.*, see footnote 1.

[5] P. L. Ricker Nos. 2640 and 2737. In Economic Collection of the Bureau of Plant Industry. Fragments of these specimens taken by the author in October, 1930, are preserved in the Mycological Collections of the Bureau of Plant Industry (Nos. 68902 and 68903).

and Moorsville, July 29, 1907, F. L. Stevens; Durham, Sept. 10, 1907, Mrs. I. W. Shields.[6] The fungus was again collected at Durham the summer of 1935 by F. A. Wolf (Fig. 1, A).

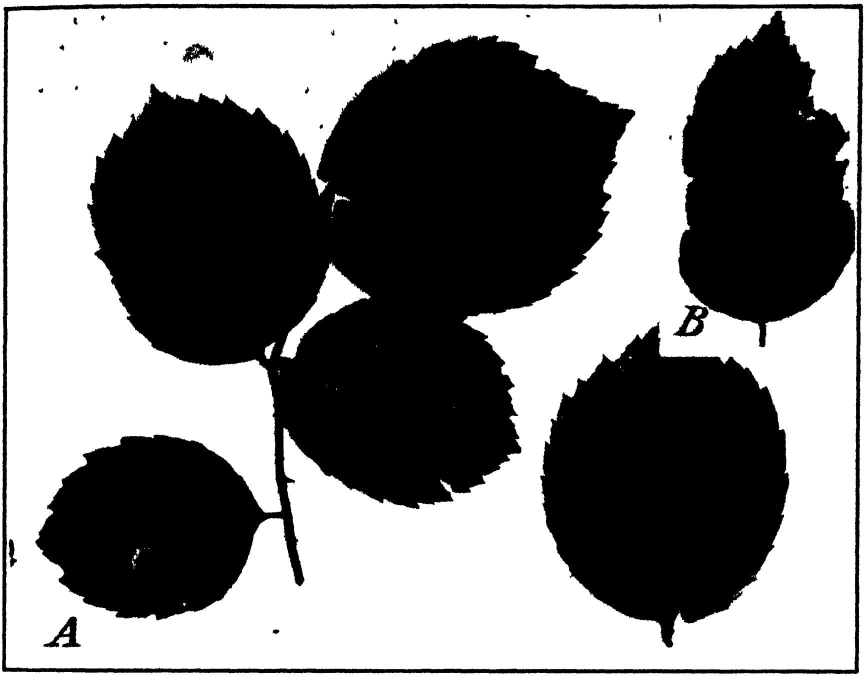

FIG. 1. A. Rose anthracnose, North Carolina. ×1. Photograph by Department of Plant Pathology, Cornell University. B. Anthracnose and black spot on same leaflet, Oregon, 1937 ×1. Photograph by McWhorter.

OREGON

In the Mycological Herbarium of the Oregon State College is a specimen from a home garden at Albany, 1902, that has been identified recently as rose anthracnose. The only earlier record of the disease in this commercial rose-growing State is from Astoria, 1898,[7] and this is based on the finding of anthracnose lesions on a phanerogamic specimen of a wild rose (*Rosa nutkana* ?) collected by the late F. V. Coville. What is believed to be the same species of wild rose is common in southern Oregon. At Roseburg, the junior writer, in 1934, observed what may be the anthracnose leaf spot on plants growing in an open woodland. During the same year he[8] definitely diagnosed as rose anthracnose a leaf spot of cultivated roses sent to the Oregon State College by a home grower in Oswego. His subsequent observations have shown that, in Oregon, the disease is very common and widespread.

[6] The specimens cited from the herbarium of F. L. Stevens have been divided, and a set has been deposited in the herbarium of the Department of Botany, University of Illinois, and in the Mycological Collections of the Bureau of Plant Industry.

[7] *Loc. cit.*, footnote 1.

[8] McWhorter, F. P. Rose anthracnose in Oregon. U. S. Dept. Agr. Plant Dis. Rep. 139, 1934.

The general occurrence of anthracnose in the rose-growing region of Portland, as well as the need for correct information relative to the disease by commercial rose growers there, was ascertained last July (July 27) when the authors visited 3 different commercial rose plantings in this district and also the municipal Rose Garden of Portland.

The first commercial planting visited consisted of 1-year plants, and the second, of 2-year plants. Each planting was in a separate field, by itself, and was grown as a crop in rotation. In the first planting there was some black spot on *Rosa multiflora,* used as stock, but no anthracnose was found. In the other, there was a small amount of anthracnose on leaves and stems of a certain variety (name unknown) budded as a tree rose.

The show garden of a commercial rose grower in another locality was next visited. There, blooming roses of numerous varieties were abundantly affected with the anthracnose. Black spot was of minor importance (Fig. 1). Representative specimens were taken from the following rose varieties: Betty, H.T.; Betty Uprichard, H.T.; Briarcliff, H.T.; Chaplin's Pink Climber, H.W.; Chatillion Rose, Poly.; C. General McArthur, Cl.H.T.; George Arends, H.T.; Golden Dawn; Golden Emblem; Golden Rapture; Hadley, H.T.; Killarney Brilliant, H.T.; Lord Charlemont, H.T.; Max Krause, H.T.; Mrs. A. R. Barraclough, H.T.; Mrs. Henry Bowles, H.T.; Mrs. Sam McGredy, H.T.; Pink Rapture; and Radiance, H.T.

The Portland Municipal Rose Garden evidently had been sprayed. In this garden rose leaf spots were not abundant, although, during the limited inspection a few anthracnose lesions were found on a rambler rose.

On September 16, at Scapoose, also in the vicinity of Portland, the junior writer made a comprehensive survey of a 2-year-old commercial planting of about 150 acres of which 70 acres were devoted to 2-year-old plants. During the entire growing season these plants were kept dusted with sulphur. Eight to 15 applications are made during the growing season. Leaf spots including those of anthracnose were entirely absent. Considering the prevalence of rose anthracnose in this region, the freedom of this planting from the disease might be taken as an indication of the control of rose anthracnose and other leaf diseases.

MICHIGAN

The occurrence of anthracnose in Michigan, alluded to elsewhere,[9] was first ascertained in 1928, when Eileen W. Erlanson sent diseased specimens from the wild rose collection of the botanical gardens of the University of Michigan to the Bureau of Plant Industry. There appears to have been no subsequent record of the disease in this State until August, 1937, when it was found on leaves of cultivated roses at Mentha by Ray Nelson and the senior writer.

[9] *Loc. cit.,* see footnote 1.

TENNESSEE

From Knoxville, Tenn., in 1934 and 1937 leaves of the Silver Moon and Van Fleet varieties, severely attacked by the anthracnose, were received from J. K. Underwood.

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INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN BROWALLIA

FRANCIS O. HOLMES

(Accepted for publication February 15, 1938)

In a preliminary test of the response of *Browallia speciosa* Hook. var. *major* Hort. to infection with tobacco-mosaic virus (tobacco virus 1) two distinct types of disease were found. In one of these, affected tissues became necrotic, but in the other they became chlorotic. The necrotic-type response was local, the other systemic. There were no intermediate types of response.

Tests with plants grown from two lots of seeds, bought in successive years, showed the presence of the two classes with regard to type of disease. In both cases there were more plants of necrotic than of chlorotic type. Only a few plants were tested from the first lot, but the second lot of seeds gave 178 plants of the necrotic type and 84 of the chlorotic type. In both tests plants of the two types were morphologically alike before infection, and could be distinguished only after inoculation.

Necrotic-type plants developed necrotic primary lesions on the second day after inoculation, and then recovered as a result of abscission of inoculated leaves. They showed no chlorotic mottling nor other evidence of systemic infection. Their subsequent growth was normal, and the injurious effect of loss of inoculated leaves was of little importance. They were regularly susceptible to infection upon reinoculation, but were able to free themselves from virus as often as it became established.

Chlorotic-type plants, on the other hand, developed no necrotic primary lesions, but suffered from a systemic disease characterized by chlorosis of the inoculated leaf and all young leaves, and a marked reduction in growth rate. If inoculated when young, they usually died as a result of the severity of the initial phase of the systemic disease. If inoculated when older, they retained more green leaf surface during the period of greatly reduced rate of growth and usually survived to produce an indefinite succession of mottled, distorted leaves and flowers.

The two types of response in *Browallia speciosa* closely resembled those already known in *Capsicum frutescens* L. This suggested that in the necrotic-type plants of *B. speciosa* there might be a single gene capable of

localizing tobacco-mosaic virus in the inoculated leaf, and of freeing the plant from this virus by leaf abscission, as had been previously shown to be the case in *C. frutescens* (1). Hybridization experiments were performed to test this hypothesis.

From the mixed stock of *Browallia speciosa* originally in hand, two pure lines, representing the two observed disease types, were produced through successive self-pollinations. These were then crossed in the following way: A homozygous necrotic-type plant, from which 120 necrotic-type and no chlorotic-type progeny plants had been derived previously by self-pollination, was used as source of pollen for hybridization with a homozygous chlorotic-type plant, from which 29 chlorotic-type and no necrotic-type progeny plants had been derived previously by self-pollination. In the F_1 generation 105 seedlings were obtained, and all were found to be of necrotic type Among these hybrid plants there was no indication of intermediate-type or chlorotic-type symptoms. This indicated complete dominance of the gene or genes introduced from the pollen parent and responsible for the necrotic type of disease.

Four F_1 plants were self-pollinated. Among the F_2 seedlings derived from them, the following ratios of necrotic-type to chlorotic-type plants were found: 52:17, 52:15, 36:13, 78:27; total 218:72, or 3.03:1. Agreement with a 3:1 ratio, implying one dominant gene controlling necrotic-type response, was satisfactory.

Backcross progeny, obtained by emasculating flowers of 12 plants of the necrotic-type F_1 generation and treating them with pollen from the recessive chlorotic-type parent plant, showed the following ratios of necrotic-type to chlorotic-type individuals: 47:37, 18:12, 14:11, 21:16, 50:46, 41:51, 37:28, 17:18, 53:53, 72:60, 12:13, and 21:17; a total of 403:362, or 1.11:1. This agreed well with expectancy on a monohybrid hypothesis.

The approximate agreement with expectation among individual ratios and totals of progenies obtained by both self-pollination and backcrossing of F_1 plants gave evidence that a single dominant gene was responsible for the necrotic type of response to infection with tobacco-mosaic virus. This dominant gene may be designated as *N* (necrotic-type response to infection with tobacco-mosaic virus), and its recessive allele as *n* (non-necrotic-type, *i.e.*, chlorotic-type, response). This gene *N* in *Browallia speciosa* is entirely comparable, both in its mode of manifestation and in its way of protecting the plant, to the gene *L* previously described in *Capsicum* (1).

Inoculated plants of a backcross set, with segregation in a 1:1 ratio, are represented in figure 1. It will be observed that the recovered necrotic-type plants, in spite of their initial loss of inoculated leaves, had outgrown the systemically infected chlorotic-type plants by the time of photographing. Healthy leaves and flowers from old, recovered, necrotic-type plants and mottled leaves and flowers from corresponding chlorotic-type plants are represented in figure 2.

The simple ratios thus far discussed, obviously pointing to a single dominant gene, controlling necrotic-type response to infection with tobacco-mosaic

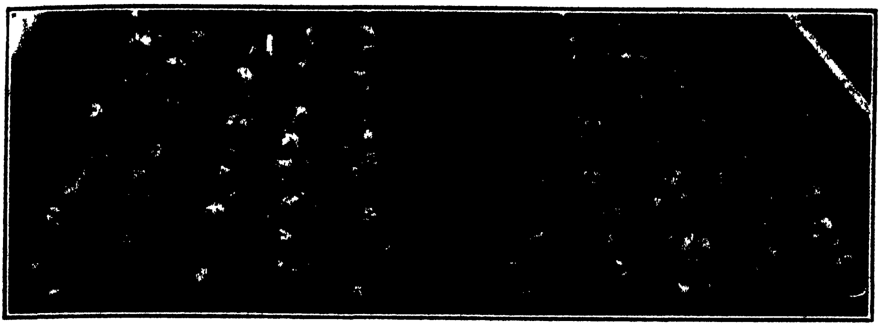

FIG. 1. Backcross progeny, of 32 diseased (*nn*) and 33 healthy (*Nn*) young plants of *Browallia speciosa* Hook., 2 weeks after inoculation with tobacco-mosaic virus. At time of photographing, the recessive-type plants (at the left) were pale and small, as a result of the severe onset of disease; the heterozygotes (at the right) were green and growing rapidly, having lost the virus through abscission of inoculated leaves. Photograph by J. A. Carlile.

virus in *Browallia speciosa,* would adequately represent the situation as known at present, had not two exceptional plants been observed in the course of these experiments. Early in the experiment that had been designed to give a pure line of necrotic-type and a pure line of chlorotic-type plants, one individual was found that gave indication of heterozygosity by segregation of the two disease types among its progeny, and yet did not give the expected 3: 1 ratio upon self-pollination. Instead it produced progeny consisting of 104 necrotic-type and 8 chlorotic-type plants (13: 1). This plant

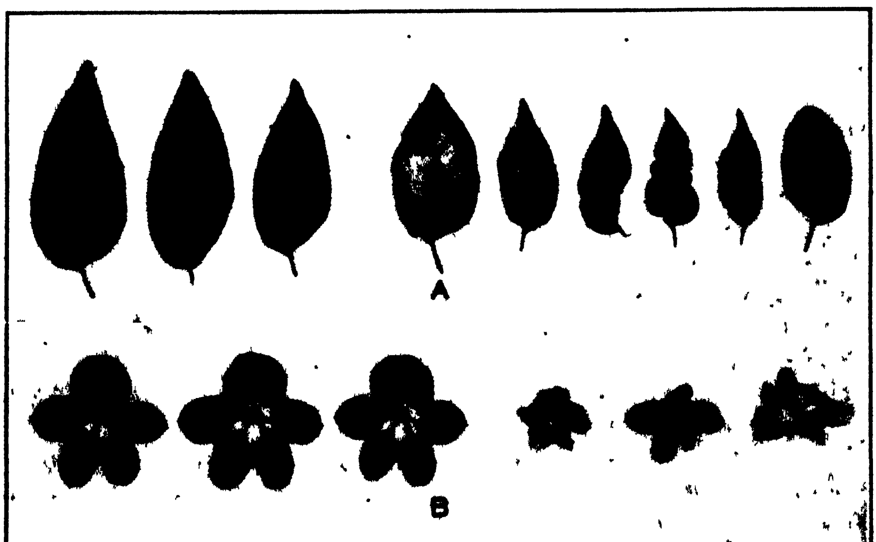

FIG. 2. Leaves and flowers from old plants of *Browallia speciosa* Hook. long after inoculation of the plants with tobacco-mosaic virus. A. Three leaves from necrotic-type (*Nn*) plants after their recovery, and 6 mottled and distorted leaves from chlorotic-type (*nn*) plants systemically affected by tobacco-mosaic disease. B. Three normal flowers and 3 mottled and distorted flowers, from similar plants of each type. Photograph by J. A. Carlile.

was not examined further, but a second plant, one of its own necrotic-type progeny, gave evidence of a similar genetic constitution by producing the same ratio (39:3) among seedlings grown from seed of a single capsule produced by self-pollination. A more thorough analysis was made of the genetic constitution of this second aberrant parent plant.

At first it seemed probable that the anomalous 13:1 ratios represented dihybrid (15:1) ratios. This possibility of a dihybrid type of inheritance was tested by crossing the second aberrant plant with the recessive, chlorotic-type plant that had been used as recessive-type parent in earlier tests. A 3:1 backcross ratio would be expected if two independently segregating dominant genes for necrotic-type response were present. In 9 backcross sets having the chlorotic-type plant as seed parent, a total of 843 seedlings were produced and tested; 433 of these were of necrotic type and 410 of chlorotic type, a ratio of 1.06:1. Confirmation of the hypothesis requiring two indistinguishable dominant genes was not given by this test, which indicated, on the contrary, that only one dominant gene was involved. In 7 sets representing the reciprocal cross, a ratio of 230 necrotic-type to 228 chlorotic-type plants was obtained. Considered together, these backcross ratios, based on more than 1300 tests, gave substantial evidence that gametes carrying the gene N and those carrying its recessive allele n were being produced in essentially equal numbers by this heterozygous necrotic-type parent, indicating only a monohybrid type of inheritance. A test of 10 of the 433 heterozygous necrotic-type plants obtained in the first-mentioned of the reciprocal backcrosses was also made by applying pollen from the chlorotic-type plant used previously; the ratios of necrotic-type to chlorotic-type plants obtained in this way were 31:22, 60:69, 60:64, 45:34, 64:67, 39:35, 42:50, 14:17, 19:19, 46:40, totals 420:417, almost exactly in 1:1 ratio. This, again, gave evidence of only one dominant gene for necrotic-type response. Backcross tests of the anomalous plants thus failed to disclose any abnormality of genetic behavior comparable to that observed in progeny obtained by self-pollination.

It seemed possible, therefore, that the discrepancy between the observed 13:1 ratio from self-pollination and a simple monohybrid 3:1 ratio might indicate the presence of a recessive zygote lethal linked with n in the heterozygous plant, but replaced in the recessive chlorotic-type parent by a pair of non-lethal alleles. The 1:1 ratios in reciprocal backcrosses had already indicated that no gamete lethal was linked with the gene n.

In order to add more data to the original observation, additional sets of plants were grown from seeds obtained from the same aberrant parent as a result of self-pollination. In the interval the parent plant had been cut back, and many new branches had grown out. The following ratios of necrotic-type to chlorotic-type plants were obtained, each representing seedlings grown from seeds of a single capsule on a separate branch of the plant: 24:9, 15:9, 33:15, 25:3, 31:16, 78:30. One of these, 25:3, resembled the original aberrant ratio of 39:3 in being much greater than a monohybrid 3:1

ratio; but all the others were considerably smaller than 3:1 ratios. Random distribution about a single mean was not indicated. This occurrence of both high and low ratios, without intermediate values, might be expected, however, if a recessive lethal gene *l* had been linked with the recessive gene *n* in some parts of this plant, but with the dominant gene *N* in other parts. Such a situation might have arisen through somatic crossing-over during the growth of the plant. If *n* and *l* had been linked originally, somatic crossing-over would cause death of all but about the same proportion of *NN* plants as had previously survived among *nn* plants.

In view of the normal ratios obtained from reciprocal backcrosses and these wholly abnormal ratios obtained from self-pollinations, it was concluded that in both aberrant plants a recessive lethal gene was linked with the recessive gene for chlorotic-type response to inoculation with tobacco-mosaic virus, and that the second aberrant plant had also, at least in some of its branches, tissues in which such a recessive lethal gene was linked with the dominant allele, which controls necrotic-type response to infection.

An attempt was made to confirm this conclusion, by further tests of the same sort. Again, some months had elapsed; the aberrant parent plant had meantime been cut back with the result that many new branches had grown out. Eight additional capsules of seeds were obtained by self-pollination of individual flowers on separate branches of this plant during August, 1937. Progenies grown from these seeds were inoculated, and later classified according to their symptoms. The observed ratios of necrotic-type to chlorotic-type plants were 86:24, 35:8, 42:15, 17:7, 53:24, 96:28, 49:24, and 24:5; total 402:135, or 2.98:1. It will be observed that there was no confirmation of previous results. No ratio of this group was as high as the aberrant high ratios previously obtained, and only three were as low as the highest among the remainder of the earlier records. Moreover, these ratios were distributed in a random manner about their mean, which agreed almost exactly with a simple monohybrid 3:1 ratio.

It is not known whether the lethal factor, for the existence of which earlier tests had given considerable evidence, had actually been lost during the continued growth of the plant, or whether its action had been temporarily nullified. Although the plant under investigation had been cut back at times and allowed to grow out anew, it had not been propagated by cuttings or grafts. All seeds representing progenies that gave evidence of the presence of a recessive lethal gene linked with the gene for type of disease were produced by pollinations at various times during the year other than summer; those giving the simple 3:1 ratios were produced by pollinations made in August. It is possible that seasonal factors may have influenced the action of the lethal gene. Since the primary purpose of the present investigation was to study the inheritance of the gene controlling disease type, and since the only available, originally aberrant plant no longer gave an anomalous response, the problem of the linked lethal gene was not pursued further.

In spite of some uncertainty regarding possible successive changes in the genetic constitution of the second aberrant heterozygous plant, all the data

taken together seem to indicate that a single dominant gene determines the necrotic type of disease in *Browallia speciosa*. All backcross data have consisted of approximate 1:1 ratios of necrotic-type to chlorotic-type plants (total 1486:1417, or 1.05:1); self-pollination of heterozygous plants has yielded data consisting either of abnormally high and abnormally low ratios (totals 168:14 and 181:79), or of simple 3:1 ratios (total 620:207, or 3.00:1). Neither the mixed class nor the simple class of self-pollination ratios would indicate more than one dominant gene for necrosis.

Except for the probable existence of a linked lethal recessive factor, therefore, the gene *N* (necrotic-type response to infection with tobacco-mosaic virus) in *Browallia speciosa* is closely comparable to the gene *L* (localization of tobacco-mosaic virus) previously described as derived from the Tabasco pepper and since established in other strains of the species *Capsicum frutescens* (2).

DISCUSSION

Chlorotic-type plants of *Browallia speciosa* were regularly affected, after a single inoculation, with a systemic chlorotic-type disease of such severity as to cause their death, or to render them practically useless for flower and seed production. On the other hand, necrotic-type plants were not seriously injured, even after repeated infection, all invaded leaves being lost by abscission a few days after inoculation.

The necrotic-type plants were consequently disease-resistant in the sense that, under the conditions of the experiments, they effectively restricted virus to inoculated leaves, and so precluded systemic manifestations of disease. It is recognized that excessively high temperatures may induce systemic necrosis in plants capable of localizing tobacco-mosaic virus at ordinary greenhouse temperatures, but such excessively high temperatures are, ordinarily, avoided as deleterious, even for healthy plants. In the present studies temperatures were thermostatically maintained between 70° and 75° F. (21° and 24° C.), so far as possible; the exceptions occurred in summer when temperatures were occasionally as high as 92° F. (33° C.).

In view of the widespread occurrence of the virus of tobacco-mosaic disease, and the ease of its unintentional transfer, it is probable that recessive-type plants may become infected at times when grown for cut flowers. It would be advantageous, therefore, to substitute a homozygous necrotic-type seed stock of *Browallia speciosa,* characterized by resistance to systemic spread of tobacco-mosaic virus, for the mixed stocks or stocks of unknown genetic constitution now in common use.

SUMMARY

Morphologically identical plants of *Browallia speciosa* var. *major* were found to fall into two categories with respect to type of disease when infected with tobacco-mosaic virus. Some plants allowed systemic spread of the virus; others effectively localized it in inoculated leaves. Systemic spread

of virus, in plants of the first class, initially caused chlorosis of young leaves and reduction of growth rate; later it induced mottling and distortion of leaves and flowers. Localization of virus in or near necrotic primary lesions, in plants of the second class, was followed by loss of all infected leaves through abscission. The necrotic-type plants were found to possess a dominant gene N in homozygous (NN) or heterozygous (Nn) condition. This dominant gene was responsible for the necrotic type of disease, and was not found in the chlorotic-type plants (nn). The homozygous necrotic-type plants constitute a sub-variety capable of recovering promptly from infection with tobacco-mosaic virus, even when inoculated repeatedly.

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LITERATURE CITED

1. HOLMES, F. O. Inheritance of ability to localize tobacco mosaic virus. Phytopath. **24**: 984–1002. 1934.
2. ———. Inheritance of resistance to tobacco-mosaic disease in the pepper. Phytopath. **27**: 637–642. 1937.

PHYTOPATHOLOGICAL NOTES

Influence of Environment, after Seedling Emergence, on Covered Smut in Barley.—In 1924 Faris[1] reported new and important results with barley covered smut (*Ustilago hordei* (Pers.) Kell. and Sw.), which have not been explained. He inoculated seed of 2 winter barleys by dusting with spores, germinated the seed under controlled soil conditions favorable to infection, and, on emergence, transplanted the seedlings to a field plot or greenhouse in late October. At maturity the following spring, the field plants were smut-free. The greenhouse plants, however, produced considerable smut. To determine the factors involved, the writer, in October, 1936, at Arlington, Va., inoculated formaldehyde-treated seed of Tennessee Winter and Wisconsin Winter barleys by dusting with spores and also by the spore-suspension method.[2] Individual seeds were uniformly sown and germinated in 2-inch pots. On emergence in mid-October, seedlings were transplanted to a greenhouse maintained at 60° F. or above, and to a field plot. In addition, seedlings were transplanted outdoors after first being held in the greenhouse for 2 or 4 weeks after emergence. The winter of 1936–1937 at Arlington, Va., was relatively mild and the field plants suffered no winter injury. The 2 varieties reacted similarly throughout the test. The average results were as follows (each of the following percentages is based upon data from approximately 200 plants): Field and greenhouse plants from noninoculated (control) seed were smut-free. When the spore-suspension method of seed inoculation was used, covered smut in greenhouse plants, and in field plants regardless of the post-emergence treatment, was high and strikingly uniform, varying only from 72.0 to 74.7 per cent (plant basis). However, when seed was inoculated by superficially blackening with spores, covered smut was: (1) relatively low (27.8 per cent) when the seedlings were placed outdoors immediately after emergence; (2) markedly increased (55.1 per cent) as a result of first subjecting the seedlings to greenhouse conditions for 2 weeks after emergence; and (3) further increased (65.3 per cent) after the seedlings were held 4 weeks in a greenhouse before transplanting outdoors. Greenhouse plants from spore-dusted seed produced 63.9 per cent covered smut.

Evidently, the placement and germination of spores beneath the hulls, as occurs in the spore-suspension method of seed inoculation, enabled the fungus, from the time of sowing to seedling emergence, to become sufficiently entrenched within the host tissues to be unaffected by the subsequent external influences. In the spore-dusting method of seed inoculation, however, the spores first must germinate after the seed is sown and the infection hyphae then must advance to the point of attack. It would appear that

[1] Faris, J. A. Physiological specialization of *Ustilago hordei*. Phytopath. 14: 537–557. 1924.

[2] Tapke, V. F. An effective and easily applied method of inoculating seed barley with covered smut. Phytopath. 25: 1038–1039. 1935.

under greenhouse conditions these delayed hyphae continued to advance in the tender, succulent tissues. On the other hand, under the rigorous field conditions of late autumn and winter and associated changes in the tissues of the barley plants, further advance of the infection hyphae was blocked and the percentage of smutted plants was greatly reduced. Whether or not this hypothesis may be proved correct, the foregoing results at least appear to indicate that if the fungus has not become well entrenched within the host tissues before seedling emergence, then relatively low temperatures and other outdoor factors immediately after emergence may play a highly important rôle in decreasing the incidence of covered smut in barley. Evidently, this was the factor involved in the above-noted experiment conducted by Faris.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

A New Pathogenically Distinct Race Derived from a Cross Between Tilletia tritici and T. levis.—The rôle of hybridization in the production of physiologic races of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn has been the subject of speculation for a number of years. That hybridization may occur between these species and between races within each species has been demonstrated by Flor[1] and later confirmed by Hanna.[2] According to Flor and Hanna the species-hybrid spores were smooth, like those of *T. levis,* but no tests for pathogenicity were reported. In Germany, Becker[3] made hybrids between physiologic races of *T. tritici* and obtained evidence of the inheritance of pathogenicity, as shown by the results of tests with the susceptible variety, Panzer. Thus far, however, no definite evidence has been presented to show that a new physiologic race of either species has been produced by hybridization.

Recently, the writer has obtained results indicating that a new physiologic race of *Tilletia tritici* has arisen from an interspecies hybrid. The physiologic races used as parents in this study were T_9 and L_8, described and numbered by Rodenhiser and Holton.[4] Hard Federation wheat seedlings were inoculated with a combination of 2 sexually compatible monosporidial lines, one of each race, according to the method described by Flor,[1] and grown to maturity in the greenhouse. Infection was obtained and the hybrid mycelium produced F_1 chlamydospores, which were reticulate, though less prominently so than those of the T_9 parent. Seed of Hard Federation was inoculated with the F_1 chlamydospores and planted in the field in the spring of 1936. A high percentage of infection was obtained, and the F_2 provided sufficient inoculum to test for pathogenicity. F_2 chlamydospores, which

[1] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis.* Jour. Agr. Res. [U. S.] 44: 49–58. 1932.

[2] Hanna, W. F. The physiology of the fungi causing bunt of wheat. 5. Pacific Sci. Cong., Canada, 1933, Proc. 4: 3195–3204. 1934.

[3] Becker, T. Untersuchungen über Sexualität bei *Tilletia tritici* (Bjerk.) Wint. im Rahmen der Immunitätszüchtung. Phytopath. Ztschr. 9: 187–228. 1936.

[4] Rodenhiser, H. A., and C. S. Holton. Physiologic races of *Tilletia tritici* and *T. levis.* Jour. Agr. Res. [U. S.] 55: 483–496. 1937.

were morphologically like those of the T_9 parent, were selected and used to inoculate seed of Hybrid 128, Oro, and Hohenheimer and, at the same time, other seed of these varieties was inoculated with chlamydospores of the two parent races. The inoculated seed was planted in the field in the fall of 1936 and smut counts were made the following summer. The bunt percentages were calculated on the basis of total and bunted heads in duplicate 6-foot rows, the number ranging from more than 500 in Hohenheimer to more than 800 in Oro and Hybrid 128 (Table 1).

TABLE 1.—*The percentages of bunt produced by races T_9 and L_8 and a hybrid between these races*

Variety	C.I. Number	Race and percentage of bunt		
		T_9	L_8	Hybrid
Hybrid 128	4512	82.0	80.0	85.0
Oro	8220	3.0	83.0	46.0
Hohenheimer	11458	28.0	0.8	16.0

The bunt percentages presented in table 1 show how distinctly T_9 and L_8 differ in pathogenicity, as well as how the hybrid differs from both parents. The variety Oro is susceptible to L_8 and resistant to T_9, while Hohenheimer is susceptible to T_9 and resistant to L_8. Each of these varieties showed a definite degree of susceptibility to the hybrid, which produced 46 and 16 per cent bunt on Oro and Hohenheimer, respectively. Thus, in view of these results, it seems probable that the hybrid inherited pathogenic properties from both parents. The hybrid was, however, less virulent on Oro and Hohenheimer than were L_8 and T_9, respectively. Nevertheless, the fact that it attacks both varieties to a significant degree distinguishes the hybrid from the parent races. There is also significance in the fact that the hybrid selection resembles *Tilletia tritici* in morphology and was able to produce 46 per cent bunt on Oro, whereas this variety is highly resistant to all of the races of this species described by Rodenhiser and Holton.[5] Furthermore, Hohenheimer is highly resistant to all of the recognized races of *T. levis* and no previously known race of either species has been able to smut both of these varieties.[5] Thus the results presented above seem to indicate that a segregate that possesses the morphology of one parent and certain pathogenic properties of both parents has been selected from a hybrid between *Tilletia tritici* and *T. levis*.—C. S. HOLTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in Cooperation with the Washington and Idaho Agricultural Experiment Stations.

Winter Injury of Buxus sempervirens.—In late December, 1928, a severe freeze following a long period of warm weather seriously damaged the large

[5] See footnote 4.

tree boxwoods (*Buxus sempervirens* L.) on one of the estates in the District of Columbia (Fig. 1). None of the other plantings, chiefly *B. sempervirens* var. *suffruticosa* L., were visibly affected by the freeze.

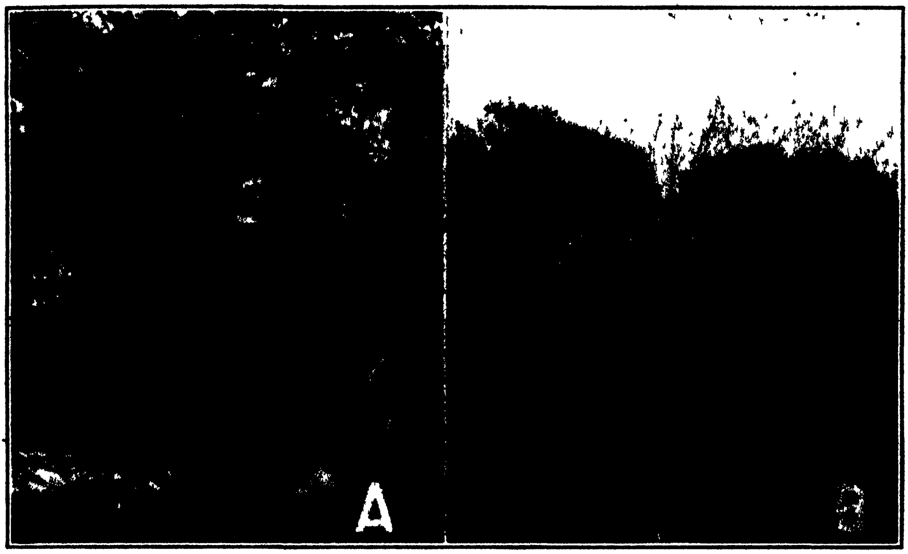

FIG. 1. A. The base of one of the injured tree boxwoods showing the effects of bark cracking and subsequent exfoliation. B. The blighted appearance of the tree boxwood on the left was caused by the effects of the bark injuries on the twigs and trunks. Photographed by G. A. Cromie.

The principal symptom of the injury was the appearance of numerous, irregular, longitudinal cracks or fissures of varying length in the bark on twigs and stems. The more severe injuries generally occurred on the trunks and resulted in a complete separation and exfoliation of the bark. There was no visible evidence of wood splitting. At the time of inspection, made only a few days after the freeze, it was observed that sap flowed freely from incisions made in the bark that was still intact. This indicated that the bark had an abnormally high sap content. In cases where most of the injured bark fell off, the trees soon wilted and died. Observations in subsequent years showed that the large bark cracks usually did not heal and the trees either ultimately died or remained in a weakened and sickly condition.

In order to make a microscopic examination of the injured tissues, discs were cut from a few of the injured stems and stained sections were made from areas that showed a stage of partial separation of the bark. The results showed that the separation was caused by a mechanical rupture that extended tangentially through the phloem and cambium. The microscopic characteristics closely resembled the type shown by plate 21, figure 3, in Grossenbacher's paper on crown-rot of fruit trees.[1] There were no visible mechanical injuries in the woody part of the stem. The individual cells in the bark

[1] Grossenbacher, J. G. Crown-rot of fruit trees: histological studies. Amer. Jour. Bot. 4: 477–512. 1917.

tissues showed no evidences of injury from the direct effects of freezing, such as wall ruptures, necrosis, and plasmolysis.

The weather records for 2 months prior to the time of injury showed that the temperature through October, November, and early December was above normal. In the later part of December there was a sudden drop in temperature, which reached 16° F. on the 9th and 20° F. on the 19th and 25th of the month. It is questionable whether these temperatures were sufficiently extreme to produce acute winter injury if the trees had hardened under normal weather conditions.—L. W. R. JACKSON, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

New Records of Anthracnose of Labrador Tea (Elsinoë ledi) and of Snowberry (Sphaceloma symphoricarpi).—In 1933,[1] anthracnose of Labrador tea was recorded in California, for the first time, through the finding of typical lesions on phanerogamic herbarium specimens of *Ledum glandulosum* Nutt. from Mendocino County (Aug., 1882, C. G. Pringle; June, 1903, Jas. McMurphy; May 26, 1913, P. Monnet). The disease is still to be found in this region, as shown by specimens collected there on July 12, 1937, by Lee Bonar (Fig. 1, A), who several years before (July 12, 1933) had found the disease in Humboldt County, adjoining Mendocino County on the north

FIG. 1. A. Anthracnose of Labrador tea, California, 1933. B. Anthracnose of snowberry, New York, 1937.

[1] Jenkins, A. E. Additional studies of species of *Elsinoë* and *Sphaceloma*. Mycologia 25: 213–220. 1933.

(Fig. 1). Specimens from both these localities were contributed by Bonar, in July, 1937.

On July 29, 1937, snowberry anthracnose was observed by the writer on *Symphoricarpus* sp. growing spontaneously along a roadside fence in open country northeast of Portland, Oregon. The existence of this disease in Oregon had not previously been known. The only other record from the far west is from California (Sonoma Co.), 1902.[2]

In New York State the disease continues to be present on the old snowberry plantings at Walton and Fishkill previously mentioned,[2] and specimens from these sources were contributed last year (Fig. 1, B). Specimens also were received from Chevy Chase, Md. (October, 1937).—ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

Effects of Bordeaux Mixture on the Control of Yellow Dwarf of Potatoes.—In the work of Black[1, 2] it was shown that the clover leaf hopper, *Aceratagallia sanguinolenta*, is apparently the only vector of yellow dwarf of the potato. An attempt was made in the summer of 1936 to find out whether control of this insect would reduce the percentage of infection. Various concentrations and amounts of Bordeaux mixture were applied to potato foliage at intervals during the season; tubers were selected at random and stored and indexed for yellow dwarf in 1937. The counts of the progeny of sprayed and nonsprayed plants were as follows: of 4813 plants sprayed with Bordeaux mixture, 14 were affected with yellow dwarf, while of 1955 nonsprayed plants, 64 were affected. The results, when treated by the X^2 test, showed a highly significant reduction in the number of yellow dwarf plants for sprayed plants when compared with nonsprayed ones. Such a reduction in the percentage of yellow dwarf (3.3 per cent to 0.3 per cent) may seem small but would be of importance to growers of seed potatoes as sufficient to permit acceptance for certification and reduce materially the labor in roguing. In case of greater spread of the disease, even greater differences might reasonably be anticipated.

Population counts showed no significant differences in the number of leaf hoppers in the sprayed and nonsprayed plots.

The experimental plots were so numerous and so thoroughly randomized that the only known difference between them was the application of Bordeaux mixture. This suggests that copper, taken up by the sprayed plants during or after spraying operations, may play a rôle in the control of yellow dwarf. During the inoculation of the plant by the insect, the presence of the copper in the plant may render the virus partially or totally noninfectious and thus account for the smaller number of plants infected with yellow dwarf.—E. O. MADER and T. C. WATKINS, Cornell University, Ithaca, N. Y.

[2] *Loc. cit.* See footnote 1.

[1] Black, L. M. Some insect and host relationships of the potato yellow dwarf virus. (Abstract) Phytopath. 26: 87. 1936.

[2] ————. The potato yellow dwarf disease. 1936. Thesis, Cornell University

BOOK REVIEWS

NIETHAMMER, ANNELIESE. *Die mikroskopischen Bodenpilze, ihre Leben, ihre Verbreitung sowie ihre oeconomische und pathogene Bedeutung*, pp. I–V, 1–193. Plates I–VI, text figures 1–47, 1937. W. Junk, The Hague, Netherlands. Den Haag, Nederland.

The author offers a brief introduction covering the universal occurrence and significance of fungi in the soil, the inadequate status of our present knowledge, and a brief presentation of the microscopic and culture methods of study. The microscopic method is regarded as ideal in theory but inadequate in practice. For culture she prefers brewery wort diluted with five times its volume of water to which she adds 5% sucrose and 3% of agar. Other media for special purposes are briefly discussed.

The text appears in 7 parts:

Systematic review of fungous groups, pp. 6–106;
Regional distribution (by nations), pp. 107–148;
Significance in nature, pp. 148–156;
Pathology, pp. 157–165;
Activities, pp. 166–176;
Growth-hormones, pp. 177–179;
Fertility relations, pp. 180–181.

In the systematic section, the species reported in the literature as isolated from soil are taken up group by group, then species by species. No diagnostic descriptions are given. No citation of place of description is given. No reference to the authority for species names or for identification is offered. The species discussed are cited from soil mycological papers without critical consideration of the validity of the use of names. For example, *Penicillium simplex*, which was in reality *Catenularia fuliginea* Saito, is cited from Janke and Holzer. Specific names have their spelling arbitrarily changed; for example, *Penicillium stolonifer* instead of *P. stoloniferum; P. terrestrum* for *P. terrestre. P. digitatum* is cited as a soil organism, but is probably rarely, if ever, found independently of the Citrus industry. *Aspergillus diversicolor* for *A. versicolor* (p. 41) is a misspelling attributable to Waksman.

Careful scrutiny of the species paragraphs suggests that names introduced by "wir" in the first line of the discussion are applied to organisms known to the author. All others appear in the third person and are probably purely bibliographic. To one familiar with soil mycological literature of the past 20 years, very few items of observation are new, but they have been collected from widely separate publications. Names listed by the various authors appear in the distribution section as reported once or more frequently in Germany or Russia or the United States. No critical examination of the use of names is reported or evident, since such examination would have excluded some usages appearing.

Section 2

In the section on regional distribution the material already used, species by species, is rearranged to produce lists of species found in particular countries. Species are grouped in relation to climate, to forest, or to meadow, without critical analysis of the reason of sources of the original lists.

The discussion of the place of particular species in nature drops the restriction to soil indicated as the purpose of the book and discusses the presence of the same fungi upon other natural substrata. Again, the species selected are those already cited as found in the soil, and other substrata are indicated as also discussed in literature.

The soil as a reservoir of plant pathogens brings forward many of the same specific names cited from the same sources.

The activities discussed for soil fungi include the breakdown of celluloses, hemicelluloses, pentosans, pectins, lignins, tannins, lipases, fermentation of sugars, and proteolytic activity.

The author explicitly disclaims completeness in presenting what is largely a compilation of existing literature. This does not justify inaccuracies in citing proper names such as Traan for Traaen; E. Lennan for Ethel McLennan; Raman for Ramann; Klebhahn for Klebahn; and there are more. These are not misprints, for they appear in the bibliography, as well as in the text.

The author has compiled a large amount of material that has been arranged arbitrarily for her purposes. For one entirely unfamiliar with soil mycology, the lists given may have some value. As a basis for a subsequent series of papers by the same author or by others, it may be convenient as a point of departure. One is compelled to doubt whether the bibliographic rearrangement of groups in *Penicillium* and *Fusarium*, many of which she has obviously never seen, has much use to those attempting to study material in culture.

Unless a proposal for reclassification is fully supported by morphological and cultural data, the user is entitled to the exact citation of at least one standard description of each species discussed.—CHARLES THOM, Principal Mycologist, Bureau of Plant Industry.

DENNIS, R. W. G. and D. G. O'BRIEN. *Boron in Agriculture.* 98 pp. Research Bulletin No. 5, West Scotland Agricultural College. Sept., 1937.

A review of the literature dealing with effects of boron on plant growth, especially crop plants in the field, is the main theme of this publication. Approximately 190 citations are given. The occurrence of this element in different plant materials, soils, rocks and minerals is given insofar as the information is available. The boron content of rocks varies, being least in igneous rocks and greatest in marine argillaceous sediments. Soils also vary but boron deficiency in soils may be primary or induced. Lime and water content of soil are important factors in the availability of this element.

Striking growth effects are described for more than 20 agricultural crop plants under field conditions. The heart and dry rot of the sugar beet with which *Phoma betae* was originally associated has definitely been shown to be due to boron deficiency. A disorder of turnips and swedes characterized by an internal browning or mottling of the flesh has been referred to by a variety of names in different countries but this author prefers to designate it as brown heart. Different organisms have been isolated from affected swedes but they now appear not to be the primary cause of the disorder. Some strains of the swede appear to manifest some resistance to brown heart but this is associated with the ability to form more extensive root systems. Tobacco is another plant belonging to a different family that manifests striking symptoms due to boron deficiency as does the tomato, another member of the same family as tobacco.

Plants belonging to the grass family appear to have a lower boron requirement than most of the other crop plants studied. However, it has been found to be essential for most grasses studied. It produces effects, according to one worker, on sugar cane that resemble Pokkah boeng disease associated with *Fusarium moniliforme* Sheldon. Excess boron has been reported to favor spot blotch (due to *Helminthosporium sativum*) on barley in sand cultures. Boron manuring has been reported to increase the resistance of wheat to rust (species not stated) and when used in small amounts in control cultures it has increased the resistance of *Agrostis tenuis* to attack of *Fusarium nivale*. The control of "Cracked stem" in celery by the use of 10 pounds of commercial borax per acre has been reported from Florida. A dressing of 30 pounds per acre was found to be toxic.

The effect of boron deficiency on the legumes is striking. Cotton grown under conditions where boron was deficient exhibited striking growth manifestations, possibly the most significant of which was shedding of flower buds and young bolls. Citrus, lettuce, and flax also manifested striking growth effects due to shortages of boron. The effect of boron deficiency on the development of the apple fruit producing what is known as drought spot and corky core have been reported from different parts of the World.

A summary table is presented for recorded symptoms due to boron deficiency with crops studied and another for quantity of boric acid, 6½ to 13 pounds per acre, or borax, 10 to 20 pounds per acre, necessary to apply in different parts of the World to prevent deficiency on the various crops previously mentioned. It is pointed out that it is difficult to obtain uniform distribution in the field of the small quantities which are required. It is to be recognized that excessive quantities of boron are toxic and, therefore, are to be avoided.—J. E. MCMURTREY, JR., Bureau of Plant Industry Washington, D. C.

OSKAR ECKSTEIN, ALBERT BRUNO and J. W. TURRENTINE with the collaboration of G. A. COWIE and G. N. HOFFER. *Potash Deficiency Symptoms.* 235 pp. 41 figs. 54 col. plates. Verlagsgesellschaft für Ackerbau M.B.H. Berlin, 1937.[1]

The student of phytopathology is now fairly well supplied with texts and monographs dealing with parasitic diseases of plants and to a lesser degree with their viroses, but there is still much to be desired in the way of usable and up-to-date discussions of the nonparasitic diseases of plants, although the latest editions of Vol. 1 of Sorauer and of Heald's Manual of Plant Diseases have improved the situation immeasurably. This lack is espe-

[1] Can be secured from B. Westerman & Co. Inc., 24 West 48th Street, New York, at $2.25 per copy.

cially notable with respect to the symptomology and pathological anatomy of plants affected by that important group of deficiency diseases resulting from the lack of or unavailability of the more common soil elements, a subject to which Sorauer devotes about 50 pages and Heald about 13 pages. The appearance, therefore, of a monograph well illustrated with black and white prints and colored plates dealing with the symptoms of potash deficiency should be welcomed by all students who desire to become more familiar with this much discussed but little understood field. The volume under discussion consists of 2 parts with concurrent German, French, and English texts, the first of which is devoted to a general discussion of potash deficiency symptoms, a detailed description of the external symptoms, and the modifications of the inner structure of typical plant organs resulting from potash hunger, and a discussion of the secondary effects of potash deficiency with reference to plant diseases, pests, and climatic factors. The second part of the book deals with symptoms of potash deficiency in specific crops and is well illustrated with photographs and with 54 colored plates prepared from color photographs and colored drawings illustrating a wide variety of normal and potash deficient crops. Included among the illustrations are a few photomicrographs illustrating the effects of potash deficiency on the anatomy of the plant. In addition to the text and numerous illustrations, a subject index and a bibliography of 209 titles also are included.

For the student or worker who would become familiar with the subject of "Potash Hunger in Plants" or who is interested in the interrelationships of potash hunger and other diseases and pests of agricultural plants, this little volume should prove an invaluable and authoritative introduction, especially since the 3 authors of the book and their 2 collaborators have devoted themselves for many years to the problems of potash nutrition. That the preparation and publication of this monograph has been inspired by the scientific bureaus of the various potash-producing syndicates is quite evident. Fortunately, the book seems free of the commercial bias that often seriously detracts from such publications.—V. H. YOUNG, Department of Plant Pathology, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

ANNOUNCEMENT

The Pacific Division of The American Phytopathological Society will hold its annual meeting in Balboa Park, San Diego, California, June 21–23, 1938. In addition to the presentation of papers, occupying four half-days, a symposium on the teaching of plant pathology will be held. An afternoon will be devoted to excursions to near-by points of interest and, following the meeting, tours to Imperial Valley or to Ensenada, Mexico, will be arranged if desired. Pathologists from all sections are invited to participate in the meetings and to present papers.—L. D. LEACH, Secretary-Treasurer, Pacific Div. of American Phytopathological Society, Davis, California.

GEORGE PERKINS CLINTON
(1867–1937)

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FLORENCE A. McCORMICK

After serving thirty-five years as Botanist of the Connecticut Experiment Station Dr. George Perkins Clinton retired from active service July 1, 1937, but he was retained on the Station staff as Consulting Botanist. While his retirement was voluntary and desired on his part, no doubt the giving up of active duties was difficult, for each morning of the short time that remained he was at his desk pursuing his usual routine. On July thirteenth he became ill and on August thirteenth he passed away. His widow, two sisters, and a brother survive. A son, the only child, was killed in France during the Great War.

Dr. Clinton was born at Polo, Illinois, May 7, 1867; but his ancestors came from Massachusetts, Connecticut, and New York. A grandfather, accompanied by his sister and their families, went west in covered wagons. Possibly as a precaution against loss of his money on the way he had invested in broadcloth which was sold to the settlers in Illinois where he established a new home. This grandfather fought in the War of 1812 and two great grandfathers served in the American Revolution.

As a child, Dr. Clinton lived the normal life of an active boy, enjoying the freedom of a small town, and having the family cow, horse, chickens, and wood-pile to give him outdoor occupation. For nearly fifty years his father was editor of the Ogle County Press, and in the newspaper rooms the son was given small tasks. There also he learned much about the process of printing and about editorial work that later was of value. In the home there was a wealth of books and magazines for old and young. In school he was studious and eager to do his best, but he was modest in accepting honors. Spelling was the one subject difficult and distasteful to him, but his determination to conquer is shown by his habit of studying that particular subject with clenched fists. From his mother he inherited his great energy. The habit of collecting may have come from the example set by his father who collected coins, stamps, and geological specimens in his earlier days and books and letters throughout his life. Probably his father's great love for flowers started the boy on his botanical career.

Dr. Clinton was graduated from the Polo High School in 1886. By this time he had already become interested in botany and through a mutual friend he met Dr. M. B. Waite who encouraged him in his decision to attend the University of Illinois and to study under Dr. T. J. Burrill. He received the degree of Bachelor of Science in 1890 and was then appointed Assistant Botanist in the Agricultural Experiment Station of the University of Illinois and Assistant in Botany in the University. His quiet enthusiasm for botany made an impression upon his associates. In 1894 he received the degree of

Master of Science from the same University. During his fourteen years of residence there, many interesting changes occurred. He entered the University before there was an Experiment Station in the State. He saw the preparations for its organization and its final establishment. At that period a botanist through training and official position was interested in all phases of the subject. Thus through his relationship with Dr. Burrill, Dr. Clinton received a breadth of training that is often lacking at the present time. With the establishment of an experiment station, parasitic fungi naturally received increased attention and Dr. Clinton became especially interested in the Ustilaginales and the Uredinales. He had published papers on both these groups while a student at the University.

During this time he began a correspondence with Professor Farlow for whom he had the highest regard in a personal, as well as in a professional way. With two friends as companions, he went from Illinois to Cambridge, Massachusetts, by bicycle, carrying with him a fresh suit in which to pay his respects to Professor Farlow. In 1900, he enrolled as a graduate student at Harvard. From that University he received the degree of Master of Science in 1901 and that of Doctor of Science in 1902.

While at Harvard, Dr. Clinton still retained his positions at the University of Illinois, but he was released from his obligations there to become Botanist at the Connecticut Agricultural Experiment Station on July 1, 1902. This position was formerly held by Professor Thaxter, the first Botanist of the Station and one of Dr. Clinton's professors at Harvard. The devotion, a kind of reverent devotion mingled with idealism, which Dr. Clinton gave to Professor Farlow, he also gave to Professor Thaxter. He had a quiet pride in occupying the position once held by the man whom he so much admired. All things at the Station that had been connected with Professor Thaxter were most carefully cherished. The old desk and most uncomfortable desk chair were retained with the conclusive explanation that they had been used by Thaxter. On the scientific side, the original potato scab culture, the old wash boiler spray outfit, devised and used by Thaxter in 1888, and some fine old drawings were among his highly prized treasures of the department. Dr. Clinton was also greatly interested in the life and poems of Cecilia Thaxter, the mother of Professor Thaxter. In 1936, he and Mrs. Clinton visited the Isle of Shoals where she had lived and is buried; and he had planned a second visit there.

Upon coming to Connecticut, Dr. Clinton entered into the agricultural activities of the State. He took a personal interest in the problems of the farmers and preferred to carry out his experiments for the control of a disease on the land where the disease was found. By that method he also came into closer contact with the grower and many of his field experiments were carried on in the days when slow and inconvenient means of travel were used by Station workers. Potato and tobacco early received his attention. In his study of *Phytophthora infestans,* he was the first to produce its oo-

spores in culture. His interest in that fungus never decreased and, only a few days before he was stricken, he spent an entire, very hot day in potato fields looking for the first appearance of late blight. This exertion probably hastened the fatal attack. He thought there was a possible connection between the potato and tomato in the transmission of the fungus and with this in mind he studied the proximity of the two crops in the field. Thus one phase of the problem, which he tried so hard to clarify, was left unsolved. He was tireless in his efforts to show growers the necessity of careful sanitation in preventing the spread of mosaic of tobacco and of the treatment of the soil of seedbed and field in combating black root rot. Years later he clearly demonstrated that the main infection of tobacco with the wild-fire organism occurs in the seedbed, which must be carefully watched and sprayed. The chestnut blight, white-pine blister rust, willow scab, and the Dutch elm disease are the major tree problems that he studied. Of outstanding importance is his work on smuts, begun at the University of Illinois and continued at Harvard. The results of this investigation are included in his Monograph of North American Ustilagineae published by the Boston Society of Natural History, and in his Ustilaginales issued in the North American Flora series. An extensive bibliography and a personal study of type specimens, as far as he was able to obtain them, and of his own collection are embodied in these publications. In the Station herbarium, the large collection of smuts, including specimens received from botanists all over the world and also specimens collected by him, testifies to the comprehensiveness of this work. Second to his interest in smuts was his general interest in rusts; and of these also there is a large collection in the Station herbarium. The department library and herbarium, housed in the same room, stand as a memorial to Dr. Clinton's industry, foresight and zeal in collecting, and are an outstanding asset of the Department as well as of the Station. The room with its excellent arrangement was planned by him. The foreign and domestic periodicals, the old and rare books, the many volumes of exsiccati, and the large collection of specimens, chiefly of fungi collected by him, were almost wholly assembled during his years at the Station. He also made a complete catalogue of all fungi mentioned in his publications. In 1935 he formally gave to the Station all his botanical books, reprints, bulletins, lantern slides, specimens, and letters from botanists all over the world. Among the letters from botanists there are interesting acknowledgments of the identification by him of hundreds of specimens of fungi.

Dr. Clinton was Botanist of the Connecticut State Board of Agriculture from 1902 until 1925, when the work of that Board was taken over by the Connecticut Department of Agriculture. He was chairman of the Committee on fungous diseases of the Connecticut Pomological Society from 1903 to 1927. A charter member of the Connecticut Asparagus Growers' Association organized in 1912, he also gave his support to that group. In the following year, when that organization became the Connecticut Vegetable Growers'

Association, he was one of the five subscribers for its incorporation. From 1919 until his death he was one of the three members of the Connecticut Tree Protection Examining Board, the first of its kind in this country. Through these diverse contacts he kept in touch with all phases of agricultural problems.

In 1904, at the request of the United State Department of Agriculture, Dr. Clinton went to Puerto Rico to study diseases of coffee. In 1908 he was called to Harvard to study fungous parasites of the brown-tail moth. The following year Harvard sent him to Japan to collect and bring back parasites for controlling the gypsy moth. In 1912 he went south to study the relationship of the chestnut blight fungus to other fungi found there. While on these missions, as well as on three trips to Europe, he collected fungi. In fact, wherever he went, for pleasure or for business, he was always collecting. From 1915 to 1926 Dr. Clinton was lecturer in forest pathology in Yale, where he was also research associate in botany from 1926 to 1929. This connection gave him an opportunity to associate with young men, in whom he was greatly interested. He was tireless in his efforts to bring the best to his students and he thoroughly enjoyed the collecting trips with his classes. His interest in his students was not limited to their work in the classroom; and they frequently came to him for consultation and advice in personal affairs.

Dr. Clinton was a fellow of the American Association for the Advancement of Science and a sustaining life member of The American Phytopathological Society, of which society he was president in 1912. He was a member of the Mycological Society of America, American Society of Plant Physiologists, Botanical Society of America, New England Botanical Society, Connecticut Botanical Society, Society of American Naturalists, Connecticut Vegetable Growers' Association, Connecticut Pomological Society, Connecticut Forestry Association, and Sigma Xi. He was made a fellow of the American Academy of Arts and Sciences in 1914 and a member of the National Academy of Sciences in 1930. In 1935 Connecticut State College gave him "Honorary Recognition" as a leader in agriculture and rural life.

As a man, Dr. Clinton has been described by his sister as "sincere, honest, generous, loyal, democratic, determined, and just." His associates also found in him all these traits in a very high degree, and to these may be added a keen sense of humor and an intense hatred for pretense of any kind. Those in sorrow caught glimpses of an unusual sympathy and his deeply religious nature. The colored man of all work who visited him during his illness and wept bitterly beside his casket remembered how often Dr. Clinton had helped him throughout the years of depression. He was comforted in his sorrow when he was asked to carry out some of the flowers and to help put back the house into its former order. The caretaker of the Station greenhouse did not want to attend the services because he felt so keenly the loss of a friend. These are tributes which testify to an innate kindness which was often con-

cealed. His frank and fearless expression of opinion were at times misunderstood but now are greatly missed and more fully appreciated.

Grateful acknowledgments are given to Mrs. Clinton; Miss Lucile Clinton, a sister; Miss Emma R. Pearson, one of his former teachers at Polo; and Dr. M. B. Waite for items about Dr. Clinton's life; to Dr. A. A. Dunlap for the photograph, which he took June 30, 1937; to Dr. Perley Spaulding for the use of his card catalogue and to the librarians of Harvard and the University of Illinois for checking over the bibliography.

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DISPERSION AND GROWTH OF BACTERIAL CELLS SUSPENDED IN AGAR

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The poured-plate technique described by Koch (9) in 1883 was the first method devised for obtaining pure cultures of bacteria from single cells. Although most investigators have accepted Koch's conclusion that each colony probably develops from a single isolated cell, other workers claim that the poured plate is not a reliable means of obtaining single-cell cultures and that pure cultures should, therefore, always be obtained by the isolation of single cells under microscopic control. Some advocates (3) of the latter method agree with Koch's conclusions but object to his method, because it furnishes no visual evidence that any particular colony developed from only one cell. Others (12, 13) believe that colonies of single-cell origin probably never are obtained in poured plates, because bacterial cells are held together by slime and because melted agar causes them to agglutinate. It even has been suggested (19) that if single cells were isolated in agar they would not grow, because single cells secured by micromanipulation usually fail to grow when placed in large quantities of broth.

Apparently, neither those who rely upon the poured plate nor those who question its reliability have tested its efficiency by direct experimentation. Studies, therefore, were undertaken to determine what proportion of the colonies of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* that developed in poured plates were of single-cell origin, what effect different techniques of

pouring the plates had on the dispersion of cells and, finally, why relatively few colonies developed on dilution plates seeded with many bacterial cells. In order to supplement the data on *P. stewarti,* somewhat less extensive studies were made on the following phytopathogenic bacteria:[1] *Erwinia carotovora* (Jones) Hol., *P. campestris* (Pam.) Bergey *et al.*, *P. phaseoli* (E.F.S.) Bergey *et al.*, *P. angulata* (F. and M.) Bergey *et al.*, *P. savastanoi* (E.F.S.) Bergey *et al.*, *P. pisi* (Sack.) Bergey *et al.*, *P. tabaca* (W. and F.) Bergey *et al.*, *P. pruni* (E.F.S.) Bergey *et al.*, *P. translucens* var. *undulosa* (E.F.S., J. and R.), *P. tumefaciens* (E.F.S. and Town.) Bergey *et al.*, *P. juglandis* (Pier.) Bergey *et al.*, *P. insidiosa* (McC.) Bergey *et al.*, *P. michiganensis* (E.F.S.) Bergey *et al.*, and *P. fascians* Tilf. Data on the dispersion and growth of the different species in agar are presented in this paper.

MATERIALS AND METHODS

The cultures used had been isolated for periods of from 1 to 3 years. Their identity was confirmed by infection of appropriate hosts. All species were typical (4) in regard to general cultural characteristics on nutrient agar and in nutrient broth except *Phytomonas fascians* and *P. savastanoi* which were of the rough-colony type. The strains of *P. stewarti* used were derived from culture B–11 described elsewhere (10). These strains differed in virulence, but all of them were typical in colony appearance except A–17, which producd dark yellow, semi-rough colonies. This culture was isolated from an inoculated plant grown in sand and deprived of nitrogen. The other strains had been derived as single-colony isolates from pure cultures.

Nutrient broth (Difco) supplemented with 0.5 per cent dextrose and adjusted to pH 6.8 to 7.0 was used in all experiments except where otherwise designated. The synthetic medium contained 0.858 g. of KH_2PO_4, 0.684 g. of $Ca(NO_3)_2 \cdot 4H_2O$, 1.75 g. of $MgSO_4 \cdot 7H_2O$, 0.37 g. of $(NH_4)_2SO_4$ and 15 g. of dextrose per liter. The ingredients were mixed and adjusted to pH 6.9 before sterilizing. All agar preparations contained 1.5 per cent of air-dry, shredded agar except where otherwise designated.

Suspensions of bacteria in agar were prepared for microscopic examination as follows. A 12- to 16-hour-old broth culture, presumably in the logarithmic phase of growth (1), was used in order to secure a high percentage of viable cells. The culture was thoroughly agitated, diluted in broth, agitated again, and then dispensed into test tubes containing melted nutrient agar at approximately 42° C. A concentration of bacteria about 1000 times that ordinarily used in poured plates was employed to facilitate counting. After the agar hardened it was sliced about 0.5 mm. thick with a flamed razor, stained for 2–5 minutes with cotton blue in lactophenol, and then washed in tap water for 15 minutes. Since the stain is specific for protoplasm, the bacterial cells were stained intensely blue, while the surrounding agar was scarcely tinted (Fig. 1).

[1] The author is indebted to W. H. Burkholder, G. C. Kent, E. E. Clayton, E. E. Wilson, J. C. Dunegan, G. L. Peltier, R. H. Bamberg, and P. E. Tilford for some of these cultures.

(*Photographs by J. A. Carlile*)

FIG. 1. Development of colonies from isolated, single cells of *Phytomonas stewarti* in poured plates. The agar from a thickly populated plate was sliced and then incubated under aseptic conditions. A, B and C. Representative fields in agar slices stained after they had been incubated for 0, 3 and 4¾ hours, respectively. Over 99 per cent of the loci occupied by bacteria immediately after the agar hardened had isolated single cells as illustrated in A, and most of these cells produced colonies as shown in B and C and Fig. 2, A and B. ×1409.

Distribution of bacteria in the agar was determined by microscopic examination of slices stained immediately after the agar hardened. The loci occupied by bacteria were classified as containing 1 isolated cell, 2 cells, or more than 2 cells. Pairs of cells attached end-to-end are listed in the tables as 2 cells, but, in the computations, they are considered as single cells, because they were obviously derived from a single cell. Pairs of cells side-by-side, end-to-side, or end-to-end with adjacent ends not in straight alignment were considered as 2 cells of different origin. Many of these pairs, however, were probably progeny of the same cell. The percentage of loci occupied by single cells was calculated and is referred to in the tables as the percentage of isolated cells. Growth of the bacteria in agar was determined by microscopic examination of agar slices stained after different periods of incubation in sterile van Tieghem cells.

EXPERIMENTAL RESULTS

Dispersion and Growth of Phytomonas stewarti in Agar. The distribution and multiplication of cells of a highly virulent strain (B–1311) of *P. stewarti* in agar were studied. Duplicate broth subcultures were dispersed into nutrient agar containing either 0.5 or 1.5 per cent dextrose. Microscopic examination showed that the bacteria were uniformly distributed in all 4 preparations. An average of 99.2 per cent of the loci occupied had single cells (Table 1) and the remainder had pairs of cells. There was no evidence of agglutination (Figs. 1, A and 2, C).

The isolated cells grew very well in agar, only 6 to 22 per cent of them failing to multiply within 12 hours (Table 1). The bacteria grew better in the 1.5 per cent dextrose agar than in the poorer medium. They multiplied more rapidly and a larger proportion of them produced colonies in this rich medium than in the poorer one.

The development of colonies from isolated cells in unstained agar preparations (0.5 per cent dextrose) was observed under oil immersion. The cells did not change perceptibly during the first hour, they began to enlarge during the second hour, and about half of them divided by the end of the third hour. The sister cells elongated immediately and subsequent divisions occurred about every 1½ hours. As the sister cells elongated, their ends slipped by each other (Fig. 1, B, a) in such a way that many of them rested side-by-side (Fig. 1, C, b) at the time of the second division. Occasionally short chains developed as the rapidly growing cells (Fig. 1, C, c) failed to complete the first division before the second one was initiated. The individual cells varied widely in their ability to start multiplying after the agar hardened (Fig. 1, B and C). The majority divided within 3 to 5 hours, but some did not divide until after about 12 hours of incubation. Some cells divided but the sister cells produced remained inactive. Most of the isolated cells, however, produced small colonies of 160 to 400 cells within 16 to 22 hours after they were placed in agar (Fig. 2, B).

The progressive changes that occur during the development of colonies

(*Photographs by J. A. Carlile*)

Fig. 2. Development of colonies from isolated, single cells of *Phytomonas stewarti* in poured plates. A and B. Representative fields in agar slices from the same preparation as in Fig. 1 that had been stained after incubation for 6 and 22 hours, respectively. C. Isolated, single cells in another preparation stained immediately after the agar hardened. A, × 1409. B and C, × 729.

may be illustrated by the data presented in table 1. In subculture B on 1.5 per cent dextrose agar, about 50 per cent of the cells divided within 2½ hours, but most of them were end-to-end and few had completed the second division. Two hours later, 86 per cent of the cells had divided and most of them were side-by-side. Many cells had divided a second or third time.

TABLE 1.—*Dispersion and growth of Phytomonas stewarti in sections of nutrient agar containing different percentages of dextrose*

Sub-culture no.	Incubation period minutes	Percentage dextrose	Distribution of bacteria in agar					
			No. of loci with				Percentage isolated cells
			1 cell	2 cells		More than 2 cells	
				End-to-end	Side-by-side		
A	0	1.5	1257	41	10	0	99.2
	210	"	202		434	2	31.7
	330	"	117		104	467	17.0
	690	"	120		22	905	11.4
B	0	1.5	585	18	5	0	99.2
	135	"	460	266	175	7	50.7
	255	"	127	183	451	138	14.1
	630	"	64		180	735	6.5
A	0	0.5	869	71	8	0	99.1
	420	"	267		290	0	47.9
	720	"	116		256	162	21.7
B	0	0.5	1031	20	7	0	99.3
	180	"	341		224	0	60.4
	740	"	163		299	263	22.5

Other subcultures of B–1311 were suspended in melted agar, as described above, and part of the agar was poured from the test tubes into Petri dishes to harden. Examinations of 7 different preparations showed an average of 99.11 ± .13 per cent of the loci occupied by single cells. There was no significant difference between the preparations allowed to harden in test tubes and those in dishes. However, when suspensions were prepared by pouring melted agar on bacteria in Petri dishes, only 98.1 and 98.6 per cent of the loci were occupied by single cells.

Studies were next undertaken to determine the distribution of bacteria in plates prepared by other methods. The effect of different nutrients in the agar, the viscosity of the agar, and the method of mixing the agar and bacteria were studied.

Poured plates containing a high percentage of single cells were prepared by several different techniques (Table 2), but the routine method described above proved to be the most reliable (No. 8). Suspensions mixed in Petri dishes (No. 3) contained almost 99 per cent isolated cells when agar of proper consistency was used. However, plates prepared with agar rendered viscous by over-cooling (No. 5) or the addition of too much agar (No. 4) had only 95 to 97 per cent of the loci occupied by isolated cells. The nutrients in the

TABLE 2.—*Effect of differences in experimental procedure upon the distribution of bacterial cells (of Phytomonas stewarti) in agar*

Experiment no.	Experimental procedure								Distribution of bacteria in agar				
	History of culture			Mixed with agar					No. of loci with				Percentage isolated cells
	Strain no.	Grown on	Diluted in broth	Composition		Temp. °C.	In dish	In test tube	1 cell	2 cells		More than 2 cells	
				Nutrient	Percentage agar					End to end	Side by-side		
Nutrient													
1	B-1311	Broth	+	None	1.5	42°	+	-	455	26	5	1	98.8
2	B-1311	Broth	+	Synth.	1.5	42°	+	-	449	21	6	1	98.5
3	B-1311	Broth	+	Nut. dex.	1.5	42°	+	-	1257	41	14	0	98.9
Agar viscosity													
4	B-1311	Broth	+	Nut. dex.	3.0	43°	+	-	774	62	47	0	94.7
5	B-1311	Broth	+	Nut. dex.	1.2	38°	+	-	720	32	23	2	96.8
Cultures used													
6	B-1311	Agar	-	Nut. dex.	1.5	42°	-	+	438	53	343	94	52.9
7	B-1311	Agar	+	Nut. dex.	1.5	42°	-	+	795	29	38	5	95.0
8	B-1311	Broth	+	Nut. dex.	1.5	42°	-	+	502	15	3	2	99.0
9	B-11-163	Broth	+	Nut. dex.	1.5	42°	-	+	984	33	8	0	99.2
10	A-17	Broth	+	Nut. dex.	1.5	42°	-	+	1276	36	13	2	98.9

agar apparently did not affect the distribution of the bacteria, since similar results were secured with bacteria suspended in plain water agar (No. 1), synthetic agar (No. 2) and nutrient-dextrose agar (No. 3). The three strains of bacteria, B-1311 (No. 8), B-11-163 (No. 9), and A-17 (No. 10), were equally well distributed in poured plates. The only method tested that could not be recommended (No. 6) consisted of scraping the surface growth from an agar slant and suspending the bacteria directly in melted agar without previously diluting them in broth. Under these conditions only 53 per cent of the loci occupied had single cells, and many clumps of cells existed in the hardened agar. However, when bacteria from agar slants were diluted in broth before they were placed in melted agar (No. 7), practically all of the groups disintegrated and at least 95 per cent of the loci occupied were seeded with single cells.

Single-cell Origin of Colonies of Phytomonas stewarti in Dilution Plates. It has been observed for other bacteria that many cells usually are sown for every colony that develops on dilution plates. This observation has been explained by assuming either that some cells failed to produce colonies (18) or that clumping of cells occurred (8). Since neither hypothesis has been supported by direct observation of the cells in agar, studies were undertaken to determine the difference between cell and colony counts for *P. stewarti* and to see if such differences were correlated with the failure of some cells to produce macroscopic colonies. A 16-hour-old broth culture of B-1311 was diluted serially and 1 cc. of each dilution was dispensed in each of 5 Petri dishes. The suspensions were mixed with melted agar at 42 to 45° C. and then incubated for 3 days at 26° C. Plates containing less than 1500 colonies were counted without the aid of a microscope, while the more thickly populated ones were observed microscopically. The number of cells present in the culture at the time the plates were poured was determined from 5 smears of .01 cc. each from both the original culture and its 10^{-1} dilution.

The data presented in table 3 show that 1 cc. of the original culture contained about 286,500,000 cells but produced only 119,400,000 colonies (calculated from the average of the 10^{-6} dilution). In other words, about 42

TABLE 3.—*The number of cells in smears from a broth culture of Phytomonas stewarti compared to the number of colonies that developed on dilution plates seeded with the same culture*

Dilution of culture	Units counted	No. of cells or colonies obtained from each cc.					
		1	2	3	4	5	Average
None	Cells ($\times 10^3$)	232,343	369,118	337,255	228,361	265,425	286,500
10^{-1}	Cells ($\times 10^3$)	24,510	27,633	31,421	26,581	29,881	28,005
10^{-2}	...	...	...	...	...	...	...
10^{-3}	Colonies	103,356	101,635	96,130	93,020	98,421	98,512
10^{-4}	Colonies	12,632	9,613	9,751	11,656	10,751	10,881
10^{-5}	Colonies	1,208	1,184	1,256	1,160	1,080	1,178
10^{-6}	Colonies	121	112	118	122	124	119
10^{-7}	Colonies	13	13	11	16	9	12
10^{-8}	Colonies	2	1	1	1	0	1

colonies developed for each 100 cells sown. Similar results were secured with a 14-hour-old culture, but a 48-hour-old culture gave only 28 colonies for each 100 cells.

After the poured plates had been incubated for 80 hours, agar that had been mixed with the 10^{-2} dilution was sliced, stained, and examined. The loci occupied by bacteria were classified according to whether they contained more or less than 16 cells, care being exercised not to count the cells that had been spread along the cut surface of the agar. Between 38 and 55 per cent of the loci occupied by bacteria in different plates contained less than 16 cells. Apparently the difference between cell and colony counts was due, for the most part, to the failure of some isolated cells, particularly those deeply embedded in agar, to develop macroscopically visible colonies.

Separation of Mixed Strains of Phytomonas stewarti on Poured Plates. The dilution-plate method should be a reliable technique for separating mixed

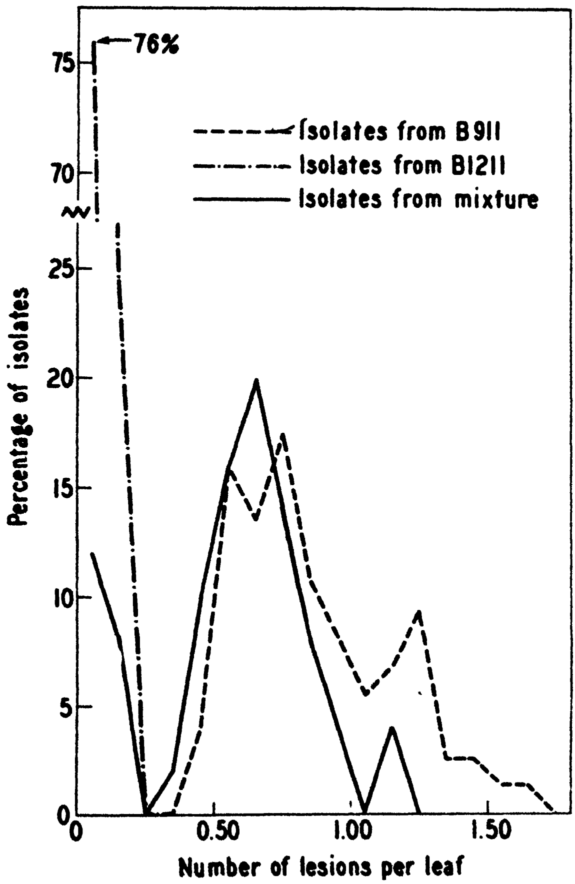

Fig. 3. Distribution of single-colony isolates from a virulent culture (B-911), a slightly virulent culture (B-1211), and a mixture of these 2 cultures. The virulence of the isolates was expressed by an infection index based upon the average number of necrotic lesions produced per leaf, and the isolates were then grouped into classes differing by 0.10 lesion. The parent types were recovered from the mixture by the dilution plate method. Seventy-four isolates of B-911, 62 of B-1211, and 45 of the mixed culture were tested.

cultures of *P. stewarti*, since almost all colonies are of single-cell origin. In order to test its reliability for separating pure lines of the bacterium, the following experiments were made. A virulent culture (B–911) and a slightly virulent one (B–1211) were seeded in separate tubes of broth, incubated for 18 hours, and then samples from the 2 were mixed in a 3rd tube. Within an hour all 3 cultures were shaken, diluted, and suspended in melted agar for dilution plates. Single-colony isolates obtained from these plates were tested for virulence. Sets of 15 to 20 sweet-corn seedlings of the variety Golden Bantam were inoculated according to the method previously described (10). The various isolates were then classified on the basis of pathogenicity, as indicated in figure 3. The data show that, although variants were present in both cultures at the time they were mixed, the isolates from the 2 fell into distinctly different classes. The isolates recovered from the mixed culture fell into either one parent class or the other and showed no tendency to form an intermediate group.

A similar experiment was made with an avirulent (B–11–163) and a highly virulent (B–1311) culture. Forty-four single-colony isolates from each parent culture and 88 from the mixture of the 2 were tested for virulence on 6-day-old sweet-corn seedlings. About 18 plants were inoculated with each isolate and the percentage of invaded leaves was computed from records taken 15 days after inoculation. The plants were then harvested and dried to constant weight at 102° C. The data obtained (Table 4) show that none

TABLE 4.—*Classification of the single-colony isolates from an avirulent culture (B–11–163), a virulent culture (B–1311), and a mixture of the two cultures according to the percentage of leaves invaded in 6-day-old sweet-corn seedlings*

Leaves invaded (Per cent)	Isolates from B–11–163		Isolates from B–1311		Isolates from mixture	
	Number	Dry weight	Number	Dry weight	Number	Dry weight
0–09	44	.299[a]	0		52	.347
10–19	0		0		0	
20–29	0		0		0	
30–39	0		7	.347	2	.265
40–49	0		3	.297	7	.246
50–59	0		6	.257	6	.237
60–69	0		17	.226	10	.206
70–79	0		9	.227	8	.159
80–89	0		1	.230	2	.170
90–99	0		1	.140	1	.100
Uninoculated controls	3	.310	3	.375	3	.327

[a] Average dry weight per plant in grams.

of the isolates from B–11–163 invaded the plants or caused a severe reduction in dry weight. The isolates from B–1311, however, invaded from 33 to 97 per cent of the leaves and caused a pronounced reduction in dry weight. Although the isolates from B–1311 differed in virulence, the least virulent one was distinctly more virulent than any of those obtained from culture B–11–163.

The mixed culture yielded 52 isolates of the B–11–163 type. None of these caused lesions or severely reduced the growth of the plants. The remaining 36 isolates were similar to those obtained from culture B–1311. Nine of them were of the weakly virulent type that invaded only 30 to 50 per cent of the leaves, while the remaining 27 isolates were of the more virulent type. These data bring additional evidence that colonies on poured plates develop from isolated, single cells.

Dispersion and Growth of Other Species in Agar. Observations similar to those described above were made on other phytopathogenic bacteria, since it was thought that the results obtained with *Phytomonas stewarti* might not apply to other species. The species listed in table 5 were tested for dispersion and growth in agar. The first 9 of these species gave results very similar to those obtained with *P. stewarti.* Over 97 per cent of the loci occupied had single cells. Some of the cultures that grew very rapidly, such as *Erwinia carotovora, P. pisi, P. angulata,* and *P. savastanoi,* had a large number of cells in late stages of division (end-to-end) at the time the agar hardened. *P. translucens* var. *undulosa* was the only one of these species in which many of the isolated cells failed to grow.

The 3 strains of *Phytomonas tumefaciens* and the cultures of *P. insidiosa, P. michiganensis,* and *P. juglandis* had 89 to 96 per cent isolated cells. These cultures also formed a few clumps of from 3 to 80 cells each. The isolated single cells from these 4 species grew readily and produced colonies within 20 hours. Many of the cells of *P. fascians,* the causal agent of sweet-pea fasciation, were clumped together in the agar and only 51 per cent of the loci occupied had single cells. This bacterium may have given results different from those secured with other species because of its different growth habits. On agar it at first produces a thin, spreading, filamentous type of growth that later becomes wrinkled and crustaceous. In broth it produces a thin, granular pellicle and many of the cells collect at the bottom of the tube in clumps; so that there is very slight, if any, clouding of the medium.

Broth suspensions of the 5 cultures that had less than 97 per cent isolated cells were examined in hanging drops. Clumps and pairs of cells were found to exist before the bacteria were placed in agar. Actual counts indicated that there were more groups of cells in the broth than in the agar mixed with the same suspensions. This difference is not considered significant, however, since it was impossible to secure reliable counts of the unstained cells while they were moving about in hanging drops. This evidence shows that agar does not cause agglutination of any of the bacteria tested.

DISCUSSION

Koch (9) stated, after he had described the poured-plate method, that he was unable to determine whether or not colonies developed from isolated cells because the thick layer of gelatine prevented microscopic examination. This handicap has been overcome by the simple methods used in these studies. Direct observations have confirmed Koch's assumption that most colonies

TABLE 5.—*Dispersion and growth of different species of phytopathogenetic bacteria in nutrient-dextrose agar*

Bacteria tested	Before incubation					After 20 hours in agar			
	No. loci with				Percentage isolated cells	No. loci with			Percentage isolated cells
	1 cell	2 cells		More than 2 cells		1 cell	2 cells	More than 2 cells	
		End-to-end	Side-by-side						
E. carotovora	803	37	17	4	97.6	2	2	742	0.3
P. campestris	1042	26	12	6	98.3	78	23	699	9.7
P. phaseoli	1323	31	21	10	97.8	88	150	418	13.4
P. angulata	853	57	16	1	98.2	12	4	751	1.6
P. savastanoi	732	109	8	5	98.5	13	8	439	2.8
P. pisi	536	176	9	1	98.6	6	1	589	1.0
P. tabaca	769	21	2	1	99.6	38	17	567	6.1
P. pruni	856	35	2	2	99.6	38	3	531	6.6
P. translucens var. *undulosa*	1013	32	8	4	98.9	644	130	217	65.0
P. tumefaciens	880	47	27	18	95.4	11	2	811	1.3
P. tumefaciens	1043	64	45	9	95.3	11	3	1055	1.0
P. tumefaciens	938	60	38	6	95.8	8	1	938	0.8
P. juglandis	602	26	30	26	91.8	31	27	705	4.1
P. insidiosa	774	63	76	25	89.3	24	77	882	2.4
P. michiganensis	937	69	59	26	92.2	27	10	956	2.7
P. fascians	252	123	0	354	51.4	50	15	383	11.2

develop from isolated, single cells. Since the effectiveness of the poured plate has been well established by 55 years of universal usage, further evidence in support of its reliability as a method for the isolation of cultures derived from single cells hardly seems necessary. The data presented in this paper are offered in the hope that they will be of use to those who may wish to properly evaluate criticisms of the method. Burri's (3) argument that the single-cell origin of any particular colony is hypothetical must be accepted, because there are a few colonies that develop from more than 1 cell. None of the data obtained, however, support those who claim that colonies rarely, if ever, develop from isolated cells.

Although several phytopathogenic bacteria have been studied, other species may give different results. Cultures that produce either heavy pellicles, strings of slime, or sediment in broth may well be suspected of containing clumps of cells. Such clumps might be avoided by using young cultures for inoculum and by using special devices (6) to shake them apart; but to be certain that they are not present, cultures should be examined in hanging drops before being dispersed in agar. According to the observations reported above, the unattached cells in broth remain separated when placed in melted agar. Although other species might be agglutinated by melted agar, no data in support of such an assumption have been found in the literature. Apparently this concept arose from a misunderstanding of Smith's (14) observation that the water of syneresis from solidified agar contained a diffusible material that caused some bacteria to agglutinate. He did not discuss bacteria in melted agar. Thermo-agglutination of bacteria has been reported (5, 2), but this phenomenon does not apply to the poured plate in which the bacteria are not exposed to a lethal temperature.

The single-cell origin of most cultures may be guaranteed by making several serial dilutions and single-colony isolations, as was done with *Phytomonas stewarti* (10). In each dilution, over 99 per cent of the loci occupied would have single cells and about 80 per cent of these cells would grow. Even if all paired cells multiplied, there would be less than 1 colony of multicellular origin for 80 of single-cell origin. After 5 successive dilutions, there would be less than 1 chance in about 3.5 billion that the culture had not been reduced to a single cell in at least one dilution. It is questionable whether even a trained observer can be any more accurate than this in determining that one, and only one, cell is floating in a hanging drop used for obtaining single-cell cultures under microscopic control.

The conclusions reached in this paper need not, necessarily, discourage the use of other pure-culture methods. There is apparently a need for both the poured-plate and other methods employing microscopic control, since each has its advantages and disadvantages. As a general rule, the poured plate will be preferred because of its simplicity. If a culture does not produce colonies of single-cell origin, it would be desirable to employ methods such as Tilford (16) used with *Phytomonas fascians*. Riker *et al.* (12, 19) obtained an occasional mixed culture of *P. tumefaciens* and *P. rhizogenes*

R., B., W., K. and S. on poured plates. It is possible that *P. rhizogenes* produced clumps of cells that involved *P. tumefaciens*, or that the strain of *P. tumefaciens* used differed from the 3 employed in these tests. Cultures of this bacterium that contain many of the star-shape bodies described by Stapp (15) might yield numerous colonies of multicellular origin.

The methods that employ microscopic control, on the other hand, also have disadvantages that have been summarized by Hort (7) and Topley, Barnard, and Wilson (17). The reliability of the method depends upon the ability of the operator to see every cell in the preparation. Since the cells are not stained, it is obvious that these methods are unsuitable for obtaining cultures of small bacteria, such as those described by Nelson (11). There would be also considerable doubt about the reliability of the method for cultures that contain involution forms. Insofar as the writer is aware, no data have been published on the reliability of the hanging-drop methods.

SUMMARY

Studies on the distribution and growth of bacteria in agar suspensions have shown that the poured-plate technique is a satisfactory method of obtaining single-cell cultures.

In plates sown with *Phytomonas stewarti*, over 99 per cent of the loci occupied by bacteria had only one cell. Eighty to 94 per cent of these isolated cells multiplied, depending on the nutrient content of the agar. The most reliable of the several methods tested for preparing poured plates with this bacterium was to suspend a young broth culture in melted agar (1.5 per cent agar at about 42° C.) and then pour the agar into a plate. Satisfactory plates were prepared, however, by several other methods in which uniform suspensions of the bacteria were placed in agar of the proper viscosity.

In plates sown with *Erwinia carotovora, Phytomonas campestris, P. phaseoli, P. angulata, P. savastanoi, P. pisi, P. tabaca, P. pruni,* or *P. translucens* var. *undulosa,* over 97 per cent of the loci occupied by bacteria had single cells. The isolated cells grew very readily in all species except *P. translucens* var. *undulosa.* In plates sown with *P. tumefaciens* (3 strains), *P. juglandis, P. insidiosa,* and *P. michiganensis,* slightly lower percentages (89 to 97) of loci were occupied by single cells. Practically all of the isolated cells multiplied. *P. fascians* was the only species studied that produced many clumps of cells in agar. Examination of broth suspensions of the 5 species that had less than 97 per cent of the loci occupied by single cells showed that the pairs and clumps of cells existed before the bacteria were placed in the melted agar.

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THE TUMOR DISEASE OF OAK AND HICKORY TREES

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INTRODUCTION

The writer's acquaintance with tumors on oak trees dates back to early 1912, when a surgeon connected with the School of Medicine of Boston University submitted a culture of a bacterial organism he had isolated from a tumor on red oak. This culture he wished to have compared with *Bacterium tumefaciens*. A report of his study of the oak disease, made to the Medical Society of Boston in 1911, stated that he believed the oak tumors to be of bacterial origin and that the organism was *Bact. tumefaciens* (6),

the cause of tumors on many varieties of plants Accompanying his culture were some photographs of large galls on oak trees.

A comparison of the surgeon's organism with *Bacterium tumefaciens* was made, as he requested, and it showed that the two organisms were unrelated In the spring of 1912, over 40 inoculations were made with his oak

FIG. 1. Galls on black oak, from which a Phomopsis was isolated, in Prince Georges County, Md.

isolation into 5 varieties of oaks, some growing out-of-doors, others in the greenhouse. No infections followed. Other plants susceptbile to *Bact. tumefaciens* were inoculated with the oak organism from Boston, with negative results. Assuming that the disease might really be due to some strain of *Bact. tumefaciens* and that the surgeon had not succeeded in isolating the infectious organism, oak trees on the grounds of the Department of Agriculture and others growing in the greenhouse were inoculated with several strains of *Bact. tumefaciens.* No galls developed on the 30 young oak twigs that were inoculated; and it was concluded that the cause of the disease must be looked for elsewhere. Two years later, when oak galls were received from several localities, they were referred to the Bureau of Entomology and Plant Quarantine. The report of that Bureau stated that the galls were not of insect origin.

As the interest in oak tumors developed, outgrowths of the same type were found on hickory (*Hicoria*) trees. It should be stated here that some time before any work was done on hickory galls the writer isolated *Bacterium tumefaciens* from a gall on the pecan, *Hicoria pecan.* Typical crown galls were reproduced by inoculations with the pecan isolation into a wide variety of plants. The hickory tree was not included, although other strains of *Bact. tumefaciens* have since been inoculated into hickory, without success. The crown gall on pecan is not at all common, and the specimen received was on a tree grown in an old peach orchard. It is probable that the orchard was well seeded with *Bact. tumefaciens.*

DESCRIPTION OF THE GALLS

The oak tumors and those of the hickory resemble crown gall in their external appearance (Fig. 1, Fig. 2, A and B). They are usually dark colored and nodular and range from 1 to 100 on a tree (Fig. 2, C). With the exception of the youngest proliferating ones, the galls are of hard wood covered with rough bark. The size varies from less than ½ in. (Fig. 3, A) to tumors over a foot in diameter (Fig. 3, B).

EXPERIMENTAL STUDIES

Attempts to Isolate Bacterium tumefaciens

Although the galls have generally been considered by pathologists and entomologists to be those of crown gall, the validity of the assumption had not been established; so research was undertaken to ascertain if a pathogen was responsible and, if so, whether the pathogen was of bacterial or fungal origin. Isolations were made from oak and hickory galls received from 7 States, including 28 lots of material, 12 of which came from Virginia and Maryland. These isolations spread over a period of years but not consecutive ones. No crown-gall colony appeared on the hundreds of plates made from young galls, nor did other bacterial colonies appear in sufficient numbers to indicate that the galls were of bacterial origin.

FIG. 2. A. Chestnut oak gall from Arlington County, Va. B. Galls on hickory near galled chestnut oak, Arlington County, Va. C. Red oak galls from Montgomery County, Md. Large galls occurred on the trunk. D. Portion of a stained section of a young chestnut oak gall. A and B. Approximately natural size. C. Much reduced. D. About × 110.

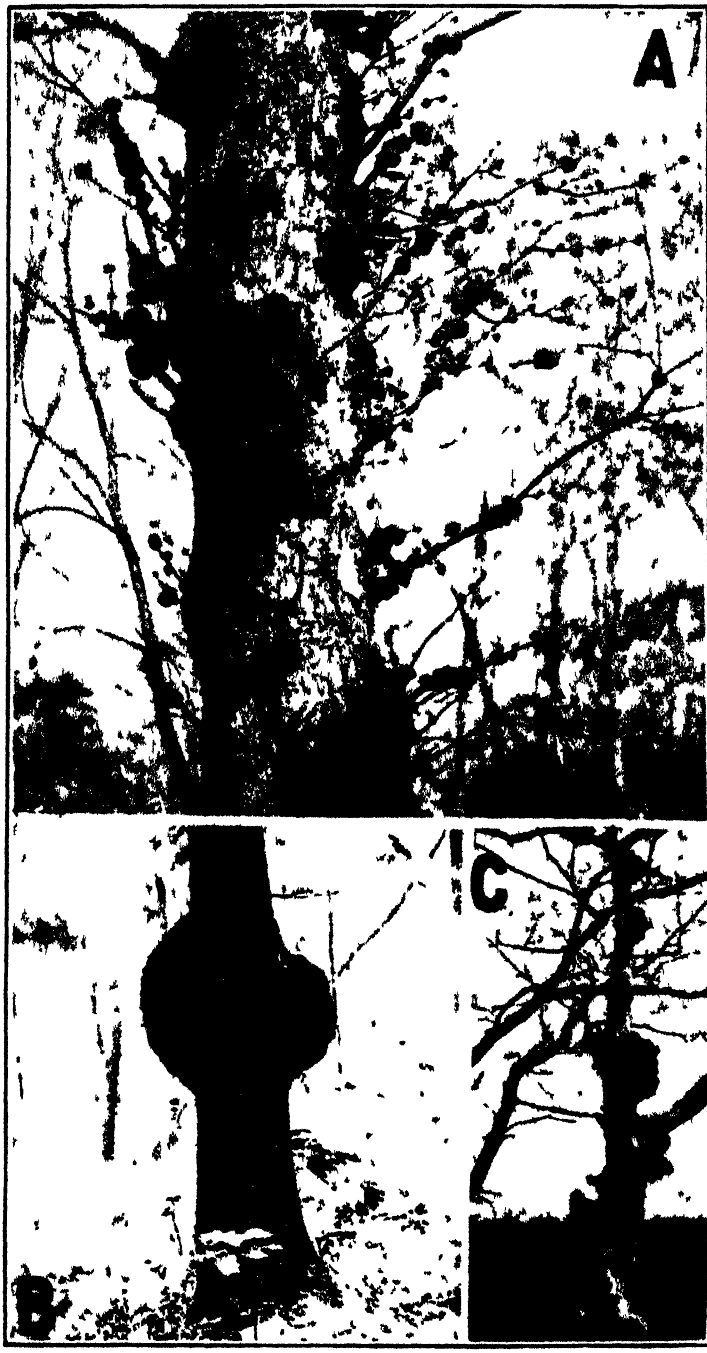

FIG 3 A Chestnut oak galls in Frederick County, Va B Large tumor on black jack oak, Arlington County, Va Circumference of gall 4 ft, 11 inches. C Red oak galls on dead trees in Massachusetts.

A Fungus Isolated from the Galls

While plating for *Bacterium tumefaciens,* fungus colonies frequently appeared and, because similar colonies grew on the plates poured from different lots of material, attention was directed to them for further study. This fungus produced pycnidia bearing tiny Phoma-like spores. In the years through which this investigation extended, intermittent though it was, this type of spore was isolated from all the galls that were studied immediately after they were taken from the trees.

Sections of galls were studied and mycelium was found in young growing galls, usually between the cells. The mature gall tissue appeared to be made up largely of xylem elements and groups of xylem cells. Part of a section of a young chestnut oak gall showing marked disarrangement of cells is shown in figure 2, D.

The Fungus Identified as a Phomopsis

Up to the present time, in the study of living oak and hickory galls, no fruiting bodies have been seen on the surface nor have the galls produced either pycnidia or perithecia when placed in a moist chamber. Galls kept out-of-doors overwinter, subjected occasionally to freezing temperatures and temperatures as low as 12° F. for short periods of time, did not produce pycnidia when brought indoors and placed in moist chambers. However, isolations made directly from the gall tissue exposed to the elements overwinter, produced pycnidia bearing *Phomopsis* spores of types *a* and *b*. Also the Phoma-like cultures kept out-of-doors overwinter and at the same temperatures as the galls mentioned above, produced both *a* and *b* types of *Phomopsis* spores when they were later placed at room temperatures. It is believed, however, that there are conditions in which pycnidia are produced on the surface of oak and hickory galls as they are on those of privet and viburnum, although the writer has not seen them.

For a number of years before the *Phomopsis* spores were obtained in culture, the Phoma-like pycnospores from various isolations were inoculated into oak and hickory stems, but nothing more than slight swellings resulted. Over 500 inoculations were made with the Phoma-like spores. With the development of the *Phomopsis* spores and better inoculation technique, the work progressed favorably.

Inoculations with Phomopsis Cultures

The *Phomopsis* spores that developed from the cultures containing Phoma-like spores proved to be infectious and produced galls on oak, hickory, *Viburnum opulus, Ligustrum vulgare, Jasminum nudiflorum* (Fig. 4, C–J) and the cultivated blueberry, *Vaccinium corymbosum.* The last 4 species are subject to a gall disease that has been reported as caused by a *Phomopsis* (1, 2, 3). Cultures of the *Phomopsis* of both oak and hickory produced galls quite readily on viburnum, privet, and jasmine; much more

Fig. 4. A and B. Beginning stages of gall on black oak trees in Prince Georges County, Maryland. Phomopsis isolated from this material. C and D. Seedling oaks in greenhouse inoculated 7–20–37 with *Phomopsis* isolated from black oak. Photographed 9–24–37. E. Privet stem inoculated with oak *Phomopsis* 5–16–36. Photographed 8–24–36. F. Stem of *Viburnum opulus* inoculated with oak *Phomopsis* 5–20–36. Photographed 11–5–36. G. *Jasmine nudiflorum* inoculated with oak *Phomopsis* 4–29–37. Photographed 9–26–37. H. Privet stem inoculated 5–22–36 with *Phomopsis* isolated from hickory gall. Photographed 8–24–36. I. *Viburnum opulus* stem inoculated with hickory gall *Phomopsis* 5–22–36. Photographed 11–5–36. J. *Jasmine nudiflorum* inoculated with hickory gall *Phomopsis* 4–12–37. Photographed 9–24–37. All slightly reduced. *Phomopsis* was reisolated from all except A and B.

readily on viburnum and privet than on either oak or hickory. It is possible that one of these shrubs may be the original host of the oak and hickory gall-producing organism. The oak *Phomopsis,* likewise, produced galls on the cultivated blueberry. The hickory strain was not tested on blueberry.

The hickory *Phomopsis* used as inoculum produced small galls on hickory and oak stems. This strain seemed to be less infectious than the one isolated from the oak. From these galls, reisolations were made and the Phoma-like type of spores was again obtained in culture. Similar reisolations from the viburnum, privet, and jasmine galls produced with both oak and hickory *Phomopsis* cultures were, likewise, made successfully. Table 1 includes inoculation records of 1936 and 1937, in which both the oak and hickory *Phomopsis* were used.

TABLE 1.—*Inoculations into various hosts with oak and hickory gall Phomopsis*

Oak Phomopsis			Hickory Phomopsis		
No. of inoculations into		No. of galls	No. of inoculations into		No. of galls
Black oak	52	16	Hickory	45	4
Chestnut oak	47	19	Chestnut oak	16	8
White oak	21	4	White oak	17	4
Red oak	4	0	Black oak	13	2
Pin oak	19	0	Pin oak	0	0
Other hosts inoculated			Other hosts inoculated		
Hickory	17	2			
Viburnum	16	14	Viburnum	12	4
Jasmine	11	7	Jasmine	6	4
Privet	23	11	Privet	13	7
Blueberry	9	4			
Total	219	77 or 35% takes	Total	124	33 or 27% takes

NOTES ON MAPLE AND ELM GALLS

Galls on maple and elm trees have been studied also. No crown-gall colonies were isolated, but the fungus producing Phoma-like pycnospores was obtained from both hosts. Inoculations with the maple isolation were made into silver, red, and ash-leaved maples in Washington, D. C., by the writer, and in Ann Arbor, Michigan, by Winifred E. B. Chase. In all, 72 inoculations were made in the spring of 1921. Some swellings resulted from the Washington inoculations but no galls. Dr. Chase's results also were negative. Some of the inoculated ash-leaved maple stems were received from her in the late summer of 1921 and, although there were no outgrowths, micro-sections through the inoculated areas showed pycnidia with the tiny Phoma-like spores. These were embedded in tissue that grew around them. Isolations were made from this material and a fungus like the original one sent to Dr. Chase developed on the plates. If the inoculation technique used in 1937 had been developed at the time the work was done on the maple seedlings and trees, the results might have been not altogether negative.

In the fall of 1937, isolations were made again from elm galls and the same fungus was obtained, as in previous years. After chilling the cultures for some weeks *Phomopsis* spores developed. In the spring of 1938, inoculations with this strain will be made into elm trees and other hosts, to try to determine its pathogenicity.

Entrance of Pathogen and Spread of Infection

The parasite apparently is a weak one, for it has to enter the stem through a wound, the tissue must be young and growing well, and the organism must have moist conditions until it becomes established. Although no pycnidia have been seen on the surface of these galls, they no doubt are formed under certain conditions. Since the enlargement of the gall results mostly through the proliferation of its outer layers of tissue, the older hyphae capable of producing fruiting bodies are not close to the surface. A live gall sooner or later should produce fruiting bodies. In cultures the spores exude from the pycnidia in long tendrils if the medium is dry, and in wide spore masses if enough moisture is present. The spores are held together by a gelatinous substance and apparently are not carried far by air currents. This factor, together with the scarcity of surface pycnidia, may explain why the disease is one of limited extent.

Throughout this investigation the writer has not found any widespread development of galls in oak and hickory trees. A thicket of trees occasionally is infected but usually the slow-growing tumors are found on a few trees or an isolated one here and there in a forest, a park, or home acreage. Hickory trees in the neighborhood of badly diseased oaks have been found galled from the surface of the ground to the topmost branches. The infection works slowly, however, and it takes years to kill a tree (Fig. 3, C). According to the farmer on whose land the tree (Fig. 1) is growing, it has been nearly dead for about ten years. Its branches still continue to bear leaves. Various species of oaks have the disease, but the writer has found the tumors more frequently on the chestnut and black oaks.[1]

Beginning stages in the growth of the gall on the main trunk of black oak are shown in figure 4, A and B. They have somewhat the appearance of young cankers. These were found on a young black oak tree growing about 15 feet from the mature infected black oak shown in figure 1. There were several branches on the young tree with this beginning stage and a few with galls an inch in diameter. Isolations were made, using the stage pictured in figure 4, A, from which a fungus with Phoma-like pycnospores was readily obtained. After chilling these cultures in the refrigerator for a few weeks, *Phomopsis* spores developed. In the spring of 1938 these cultures will be used for inoculations into susceptible hosts.

The slow growth of the galls and the slow spread of the infection contribute to an easy control of the disease by simply cutting and burning the diseased parts or destroying the whole tree.

[1] In the spring of 1938 leaves developed on some branches but were dead by the middle of May.

PERFECT STAGE OF THE PHOMOPSIS NOT OBTAINED

Up to the present, the perfect stage of the fungus has not been found. Repeated attempts to produce this stage were made by placing galled stems in wire screening on the ground under oak trees and covering with oak leaves; by sinking wire baskets containing galls in snow; by leaving water-proof-covered cultures exposed to winter temperatures from one to several months; by culturing the fungus in a wide variety of artificial media; by culturing on sterile oak leaves and stems in tubes; by sterilizing oak and hickory stems in mercuric chloride 1 to 1000 for 10 minutes, washing in sterile water, inoculating with various strains of *Phomopsis* and placing them in sterile test tubes; by inoculating oak bark, both sterilized and nonsterilized; by streaking corn meal agar plates with different strains of *Phomopsis*, as oak and hickory, oak and viburnum, etc., with the hope of obtaining + and – strains with the production of perithecia; by subjecting cultures alternately to warm and then cold temperatures, and *vice versa;* and by drying cultures, then moistening them, and *vice versa.* Galls were taken from the trees at different times of the year and examined, but no perithecium was found that could be considered related to *Phomopsis.*

GALLS AND CANKERS OF OAK NOT MANIFESTATION OF THE SAME DISEASE

In the tumor disease of oak and hickory there is a distinct outgrowth. The canker diseases of oak described in literature need not be confused with it. The goitre disease of oak, pine and fir, occurring in Germany, however, may be the same or a related disease. In the latter, the outgrowths are stated to be of considerable size and either remain covered with more or less normal bark or become a broken-down canker to which wound organisms have access.

Sprengel (7, 8), who is studying goitre, makes the statement that there is no clue to the cause, but most of the evidence indicates it may be due to an hereditary predisposition of certain trees to produce tumors in response to certain environmental factors.

Harshberger (5) refers to tumors on oaks as large as a man's head or a large pumpkin, caused by the fungus *Dichaena strumosa.* A letter from Dr. Harshberger, written March, 1922, in response to an inquiry by the writer, stated that his authority for the statement in his book was J. B. Ellis of New Jersey. In *North American Pyrenomycetes,* Ellis (4) states that oak galls caused by *Dichaena strumosa* Fries, are common on limbs and trunks of living *Quercus coccinea* and *Q. nigra,* in Carolina (Ravenel), Mexico, and New Jersey (Ellis), and are very injurious, finally killing the trees. He describes the perithecia of *D. strumosa,* but ends in stating that "We have never found ascigerous specimens of this or *D. faginea.*" The writer hopes some day to find perithecia of a *Diaporthe* on the oak and hickory tumors and, through culturing the ascospores, prove the *Phomopsis* of these galls is associated with it.

Some comparative studies have been made of the different gall-producing strains of *Phomopsis* as to colony and pycnidia formation and size of *a* and *b* spores, but much remains to be done. These comparisons will be reported later.

SUMMARY

The tumor disease of oak and hickory is caused by a parasite, the fungus, *Phomopsis*.

The two strains are cross inoculable. They are also pathogenic to and produce galls more readily on viburnum, privet, and jasmine.

Three types of pycnospores are produced. In isolating from a gall taken directly from a tree, Phoma-like spores are formed first. Later, after chilling, these cultures produce *Phomopsis* spores of *a* and *b* types.

Up to the present, no perfect stage of the fungus has been found in nature or in culture.

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THE RUST OF STONE FRUITS[1]

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INTRODUCTION

The rust of stone fruits, caused by *Tranzschelia pruni-spinosae* (Pers.) Diet.,[2] is of considerable economic importance in many parts of the world. It was reported from Australia (24) in 1890 and subsequently by McAlpine (21, 22, 23) and numerous later investigators. Cunningham (8) has discussed its occurrence in New Zealand, and Fikry (12) recently has studied its

[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station. This is abridged from a thesis presented to the Faculty of the Graduate School of the University of Arkansas in partial fulfillment of the requirements for the degree of Master of Science.

[2] This fungus is also called *Puccinia pruni-spinosae* Pers. by some investigators.

prevalence in relation to the rise and fall of the water table in the Nile delta of Egypt. Butler and Bisby (5) report its occurrence in India, and it is known to be widely distributed in Europe and North America. In addition typical specimens have been examined from China, Japan, Java, Uganda, Colombia, Venezuela, Brazil, Uruguay and the islands of the West Indies.

In the southeastern portion of the United States the disease rarely appears before midsummer and is confined entirely to the leaves. Since fruit and twig infections are unknown in this section, a premature defoliation is the only visible effect of the disease. In the western portion of the country, on the other hand, Pierce (26), Goldsworthy and Smith (15), and others have reported that it frequently appears a few weeks after the leaf buds unfold. These early spring infections are derived from overwintered lesions on the twigs and, once established on the leaves, the disease is commonly present throughout the growing season. Moreover, under certain environmental conditions, the fungus attacks the fruit and causes serious financial losses.

In Europe the disease does not appear until mid-summer, according to Brooks (4) and Ducomet (9); while in Australia and New Zealand, McAlpine (22) and Cunningham (8) have reported serious injury to the fruit, as well as premature defoliation.

In 1925 the writer began a study of the rust fungi found on various species of *Prunus* to determine why there were such marked variations in the behavior and importance of the disease. While the results furnish only a partial explanation of this variation, the demonstration of the difference, hitherto not clearly realized, between the forms of *Tranzschelia pruni-spinosae* attacking the wild and cultivated species, the resultant clarification of host relations, and the revision of the present taxonomic concept of the causal organism are all related problems whose solution will aid indirectly in explaining the variations noted in the behavior and importance of this rust.

MATERIALS AND METHODS

The studies herein reported may be divided into two parts. First, a series of inoculation experiments with urediospores were performed over a period of years to ascertain the interrelation between the forms on various cultivated species and between those attacking the cultivated and wild species. In addition, the inoculation experiments with aeciospores performed by other investigators were repeated to determine more precisely the relation between the aecial stages on various species of Ranunculaceae and the uredial and telial stages on wild and cultivated species of *Prunus*. Since these inoculation experiments involved the use of living material from various parts of the country, they were carried out in Petri dishes, with the inoculated leaves floating on sugar solutions, to eliminate the possibility of introducing infectious material into Georgia and Arkansas orchards.

In the second part a detailed examination was made of 261 specimens of *Tranzschelia pruni-spinosae* from the Division of Mycology and Disease Survey, United States Department of Agriculture, as well as 128 additional

specimens in the writer's private herbarium. The host, locality, collector, and date of collection shown on the herbarium label were recorded for all collections and a slide was prepared from each collection showing teliospores. From these data it was possible to make certain groupings of the hosts and arrive at conclusions concerning host relations that would not be evident from the casual examination of isolated specimens.

RESULTS

Inoculation Experiments with Urediospores

In 1905 Arthur (1) was unable to infect peach, *Prunus persica* S. & Z., even ". . . under seemingly the most favorable conditions . . ." with urediospores from wild cherry, *P. serotina* Ehrh. leaves. During the period from 1925 to 1927 the writer obtained similar results at Fort Valley, Georgia, and found also that the urediospores produced on peach leaves would not infect the leaves of wild cherry, *P. serotina.*

During the period 1935–1937 urediospores from various wild and cultivated hosts were used in inoculation experiments at Fayetteville, Arkansas. The results (Table 1) of these experiments confirm the previous experiments of Arthur (1) and the writer and prove that 2 forms of the rust exist. One form is able to infect only the cultivated species and the other attacks only the wild species. Since the peach was successfully inoculated in Arkansas with urediospores from a number of cultivated species from Georgia, Texas, California, and Oregon it is evident that the form attacking the cultivated species is widely distributed throughout the United States.

Inoculation Experiments with Aeciospores

Additional evidence that there are 2 distinct forms of the rust fungus on the genus *Prunus* was obtained in the inoculation experiments with aeciospores from the various alternate hosts. In 1905–06 Arthur (1, 2) successfully inoculated *Prunus serotina* and *P. pumila* L.[3] with aeciospores of *Aecidium hepaticatum* Schw., from *Hepatica acutiloba* DC. (*H. acuta* (Pursh) Britton), but failed to infect *P. americana* Marsh., *P. cerasus* L. and *P. persica,* leaves with these aeciospores. In commenting upon these results he stated,

> ". . . It is not possible to state what significance is to be attached to the failure to infect peach, plum, and cultivated cherry with spores that readily infect the wild cherry . . .",

and later, Jackson (17) suggested that these results indicated the presence of distinct strains of the fungus in the United States.

Clinton and McCormick (6) and the writer (Table 2) successfully inoculated wild cherry, *P. serotina,* leaves with aeciospores from *Anemone quinquefolia* L. and *A. caroliniana* Walt., respectively, but were unable to infect peach

[3] In his latest publication Arthur (3) has substituted *Prunus nana* for *P. pumila* in the report of these experiments. No authority is given for the binomial *P. nana* but it is inferred that he used *P. nana* Du Roi (= *P. virginiana* L.) in these experiments.

TABLE 1.—*Inoculation experiments with urediospores of Tranzschelia pruni-spinosae at Fayetteville, Ark., 1935–37*

Date	Inoculum		Host inoculated	Results
	Host	Source		
April 12, 1935	*Prunus serotina*	Arkansas	*Prunus serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
April 25, 1935	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
June 3, 1935	do.	do.	do.	Negative
do.	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. angustifolia* Marsh.	No results, leaves molded
June 7, 1935	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
do.	do.	do.	*P. communis*	Negative
August 5, 1935	*P. munsoniana* W. & H.	do.	*P. munsoniana*	Positive, uredia produced
do.	do.	do.	*P. persica*	Negative
Sept. 5, 1935	*P. persica*	Texas	*P. persica*	Positive, uredia produced
do.	do.	do.	*P. serotina*	Negative
do.	do.	do.	*P. munsoniana*	Negative
Oct. 21, 1935	do.	Georgia	*P. persica*	Positive, uredia produced
do.	*P. davidiana* Franch.	do.	do.	Positive, do.
March 12, 1936	*P. communis* Fritsch.	California	*P. persica*	Positive, do.
May 6, 1936	*P. serotina*	Arkansas	*P. serotina*	Positive, do.
May 13, 1936	do.	do.	*P. munsoniana*	Positive, do.
May 15, 1936	*P. armeniaca* L.	California	*P. persica*	Positive, do.
May 21, 1936	*P. communis*	do.	do.	Positive, do.
do.	do.	do.	*P. munsoniana*	Negative
do.	*P. munsoniana*	Arkansas	*P. persica*	Negative
do.	do.	do.	*P. munsoniana*	Positive, uredia produced
April 20, 1937	*P. persica*	California	*P. persica*	Positive, uredia produced
Sept. 14, 1937	*P. domestica* L.	do.	do.	Positive, do.
Sept. 29, 1937	do.	Oregon	do.	Positive, do.

leaves with aeciospores from these hosts. In 1931 Scott and Stout (28) reported the successful inoculation of various varieties of almond, apricot, cherry, nectarine, peach, plum, and prune, all cultivated species of *Prunus*, with aeciospores from *Anemone coronaria* L., a plant of European origin. They did not inoculate wild species of *Prunus*, but, in 1935, the writer obtained rust-infected *A. coronaria* plants[4] from California and inoculated the leaves of the peach and wild cherry, *Prunus serotina*, with aeciospores from this material (Table 2). Urediospores developed on the peach leaves but no infections were secured on the leaves of the wild cherry. These experiments were limited in number but confirmed Scott and Stout's results with respect to the peach and showed also that the aeciospores from *A. coronaria* will not infect the leaves of wild cherry.

Examination of Herbarium Material

The 389 collections (Table 3) of uredial and telial stages examined consisted of specimens of 26 species and 3 varieties of *Prunus*, both cultivated and wild, from various parts of the world, as well as a number of specimens in which the host was not identified.

No constant differences were noted between the uredial stages on the wild and cultivated species of *Prunus* in the United States. In general, the uredia are pulverulent, cinnamon-brown, and usually are formed on the dorsal surface of the leaves, although occasional specimens are encountered in which the sori occur on both surfaces. The urediospores are oblong-clavate or oblong-fusiform and measure 15–23 by 28–42 μ. The wall, which is 1 to 1.5 μ thick at the sides, is thickened at the apex. It is smooth and brownish-yellow at the apex but becomes echinulate and paler toward the base of the spore. The pores, which vary from 3 to 5 in number, may be equatorial but frequently, in the longer spores, are in the upper third. Capitate paraphyses are intermixed with the urediospores in the sori.

In contrast to the general uniformity of the urediospores produced on the various hosts the writer (10) found that the teliospores produced on the cultivated species can be distinguished readily, both macroscopically and microscopically, from the teliospores produced on the leaves of the wild species.

The teliospores (Fig. 1, A–E) produced on the wild species are bicellular, oblong or obovate-oblong, and measure 18–27 by 30–39 μ. The spores consist of 2 globoid or oblong-globoid cells, with chestnut-brown walls 1.5–2.5 μ thick, coarsely verrucose over the entire surface of both cells. Those (Fig. 1, F–I) produced by the fungus attacking the cultivated species are bicellular, dark chestnut-brown, with the upper cell globoid and the lower varying from globoid to irregular-shape forms, definitely contracted at the base. The wall of the upper cell is generally but not always thickened at the apex. It is much darker than the wall of the basal cell and is coarsely verrucose over its entire surface. The wall of the basal cell is never completely verrucose and, in some

[4] These plants were secured through the kindness of F. J. March and G. L. Stout, of the California State Department of Agriculture.

TABLE 2.—*Inoculation experiments with aeciospores of Tranzschelia pruni-spinosae at Fayetteville, Arkansas, 1935–36*

Date	Inoculum		Source	Host inoculated	Results
	Species	Host			
March 29, 1935	*Aecidium hepaticatum*	*Anemone caroliniana*	Arkansas	*Prunus serotina*	Positive, uredia produced
do.	do.	do.	do.	*P. persica*	Negative
April 6, 1935	do.	do.	do.	*P. serotina*	Positive, uredia produced
do.	do.	do.	do.	*P. persica*	Negative
April 12, 1935	*Aecidium punctatum* Pers.	*A. coronaria*	California	*P. persica*	Positive, uredia produced
do.	do.	do.	do.	*P. serotina*	Negative
April 30, 1935	do.	do.	do.	*P. persica*	Positive, uredia produced
April 26, 1936	*Aecidium hepaticatum*	*A. caroliniana*	Arkansas	*P. persica*	Negative
do.	do.	do.	do.	*P. serotina*	Positive, uredia produced
April 27, 1936	do.	do.	do.	*P. persica*	Negative
do.	do.	do.	do.	*P. munsoniana*	Positive, uredia & telia produced
do.	do.	do.	do.	*P. serotina*	Positive, uredia only
May 5, 1936	do.	do.	do.	*P. persica*	Negative
do.	do.	do.	do.	*P. serotina*	Positive, uredia only

TABLE 3.—*Enumeration of specimens examined arranged according to host species and geographical distribution. The first number opposite any species is the number of specimens on which teliospores were found; the second number indicates the number of specimens examined that did not have teliospores on them*

Host	North America	South America	Europe	Asia	Africa	Australasia
Prunus americana	23–0					
angustifolia	7–1					
armeniaca	2–8		3–1			0–1
" var. *anzu* Maxim.				1–0		
avium L			1–0			
besseyi Bailey	5–1					
capuli Cav		0–1				
cerasifera Ehrh		1–0				
" var *pissardii* Koehne			1–0			
chicasa Michx.	3–1					
communis	1–3		1–0	2–1		1–0
davidiana	3–2					
domestica	15–0		29–0			
" var *insititia* Bailey			3–0			
grayana Maxim				1–0		
hortulana Bailey	7–0					
japonica Thunb		1–0				
mahaleb L			2–0			
mexicana Wats	3–0					
mume S & Z	2–0			1–0		
orthosepala Koehne	1–0					
pennsylvanica L	2–0					
persica	14–93	0–19	1–4	0–3	2–2	3–3
pumila L	3–0					
serotina	25–14					
simonii Carr.	1–0					
spinosa L.	1–0	1–0	16–0			
virginiana L	4–1					
watsonii Sarg.	1–0					
sp.	20–5	2–1	5–1		2–0	

specimens, appears almost entirely smooth The spores measure 15–23 by 26–39 μ. The basal cell generally is narrower than the apical cell

Macroscopically the teliospores on the leaves of the wild species appear as pulverulent, chestnut-brown masses, while those on the cultivated species appear as compact, black clusters The 2 types can be distinguished readily by these differences.

In 1901 Jacky (18) observed that the rust fungus attacking certain cultivated hosts in Germany produced teliospores that differ from those originally described by Persoon. Fischer (13) confirmed these observations and proposed that the teliospores produced by the form representing Persoon's species should be designated as the *forma typica* and the type discovered by

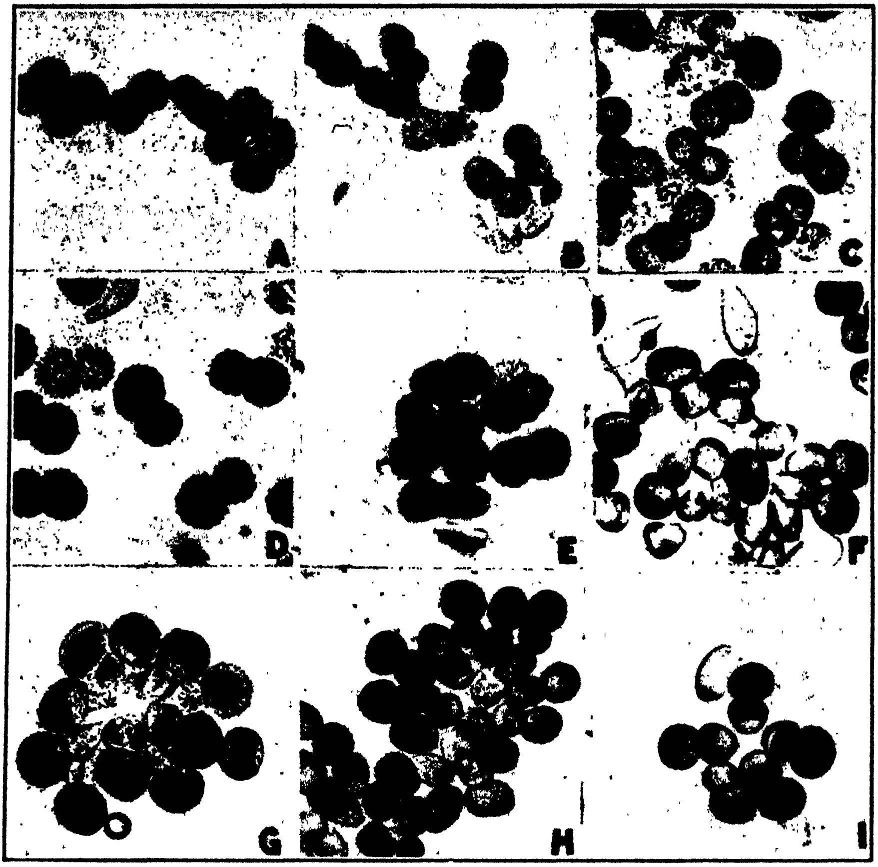

FIG. 1. Teliospores of *Tranzschelia pruni-spinosae* (Pers.) Diet., from the leaves of various species of *Prunus* in the United States. A–E. Examples of the variety *typica*. A. From *Prunus chicasa* Michx., Kansas, Sydow "Uredineen" 1179. B. From *P. besseyi* Bailey, Nebraska, J. M. Bates 451. C. From *P. hortulana* Bailey, Arkansas, Bartholomew "Fungi Columbiani" 2887. D. From *P. angustifolia* Marsh., Georgia, Dunegan 642. E. From *P. americana* Marsh., Texas, S. E. Wolff 198. F–I. Examples of the variety *discolor*. F. From *P. persica* S. & Z., Texas, Dunegan 875. G. From *P. domestica* L., California, D. W. Coquillet. H. From *P. davidiana* Franch., Georgia, Dunegan 835. I. From *P. mexicana* Wats., Georgia, Dunegan 834. All fixures × 270.

Jacky as the *forma discolor.* Subsequently, Kreig (19), Linsbauer (20), Eriksson (11), and Salmon and Ware (27) also recognized the occurrence of 2 types of teliospores in Europe. Grove (16), however, did not accept Fischer's proposals and stated that these differences in teliospores are ". . . hardly worthy of mention. . . ."

When the teliospores produced on the various species of *Prunus* in the United States were compared with European material, it was evident that those from the wild species in the United States were similar to the teliospores (Fig. 2, A–C) designated as the *forma typica* in Europe. The teliospores from the cultivated species in the United States were similar to those (Fig. 2, D–F) designated as the *forma discolor* in Europe. As a matter of conveni-

ence these 2 forms will be referred to in the following pages as the *typica* and *discolor* types, respectively.

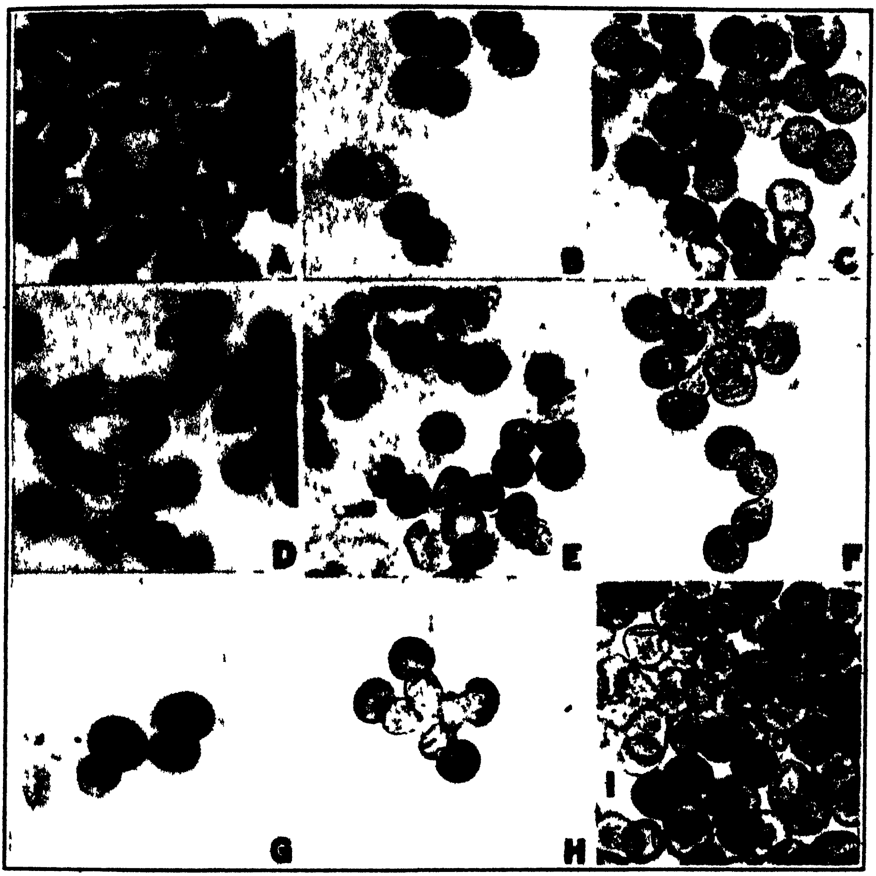

FIG. 2. Teliospores of *Tranzschelia pruni spinosae* produced on various species of *Prunus* throughout the world, exclusive of United States. A–C Examples of the variety *typica*. A. From *Prunus domestica*, Latvia, Kirulis "Fungi Latvici." B. From *P. domestica*, Austria, Petrak "Fungi Eichleriani" 192. C. From *P. spinosa*, Austria, Bresadola 7087. D–H. Examples of the variety *discolor*. D. From *P. domestica*, Italy, Bresadola. E. From *P. armeniaca*, Austria, de Thumen "Mycotheca Universalis" 1238. F. From *P. avium*, Spain, Caballero. G. From *P. communis*, Palestine, A. Aeronson "Fungi of Palestine" 60. H. From *P. spinosa*, Uruguay, G. Herter "Plantae Uruguayenses Exsiccatae" 1261. I. Teliospores from type material of Fuckel's *Puccinia discolor* (Fungi Rhenani 2121). All figures ×270.

In general, the *discolor* type of teliospore occurs almost entirely on the cultivated species, while the *typica* type predominates on the wild species; but, when the various specimens examined are arranged (Tables 4 and 5) according to the type of host (*i.e.* cultivated or wild), certain exceptions become evident. Thus some collections of *Prunus besseyi*, *P. hortulana*, and *P. mexicana*, wild species, had the *discolor* type teliospores on them. On the other hand, *P. armeniaca* and *P. domestica*, cultivated introduced species, were infected in a few instances by the form producing the *typica* type of

teliospores. Jacky (18) and others have observed a very similar situation. *Prunus spinosa* and *P. domestica*, normally infected in Europe with the form producing the *typica* type of teliospore, occasionally are infected with the *discolor* type. In the 29 *P. domestica* specimens of European origin examined by the writer, the *discolor* type of teliospore was found on 8 and the *typica* type on 21 specimens. Of the 15 *P. domestica* specimens examined from North America, the *typica* type of teliospore was found on only 2, while the *discolor* type was found on the remaining 13.

Since the genus *Prunus* is not indigenous in the Southern Hemisphere (except in the mountains of northern South America) the absence of the *typica* type in Africa and Australasia is not unexpected. The *discolor* and *typica* types were found on the few specimens available from Asia (Fig. 2, G), but only the *discolor* type (Fig. 2, H) was found on the specimens from South America.

TABLE 4.—*The type of teliospore found on various species of Prunus in North America*

Host	Type of host	Number of collections	Number of collections showing	
			discolor type teliospores	*typica* type teliospores
Prunus americana	wild	23	0	23
angustifolia	do.	8	0	7
armeniaca	cultivated	10	1	1
besseyi	wild	6	1	4
chicasa	do.	4	0	3
communis	cultivated	4	1	0
davidiana	do.	5	3	0
domestica	do.	15	13	2
hortulana	wild	7	1	6
mexicana	do.	3	2	1
mume	cultivated	2	2	0
orthosepala	wild	1	0	1
pennsylvanica	do.	2	0	2
persica	cultivated	107	14	0
pumila	wild	3	0	3
serotina	do.	39	0	25
simonii	do.	1	0	1
spinosa	cultivated	1	0	1
virginiana	wild	5	0	4
watsonii	do.	1	0	1
sp.		25	3	17

DISCUSSION

The results of the inoculation experiments and the study of the herbarium specimens clearly indicate that there are 2 distinct forms of the rust fungus attacking the genus *Prunus* throughout the world.

These results do not explain the variations noted in the prevalence and importance of the disease on the cultivated hosts. However, since the peach, as well as most of the other cultivated species, is infected only by the *discolor* type, these variations at least cannot be attributed to the existence of different species of rust fungi attacking the peach in various parts of the world.

TABLE 5.—*The type of teliospore of Tranzschelia pruni-spinosae found on species of Prunus in various parts of the world exclusive of North America*

Host	Type of host	South America *discolor* type	South America *typica* type	Europe *discolor* type	Europe *typica* type	Asia *discolor* type	Asia *typica* type	Africa *discolor* type	Africa *typica* type	Australasia *discolor* type	Australasia *typica* type
Prunus armeniaca	cultivated			2	1						
" var. *anzu*	do.					0	1				
avium	do.			1							
cerasifera	do.	1	0								
" var. *pissardii*	do.			0	1						
communis	do.			1	0	2	0			1	0
domestica	do.			8	21						
" var. *insititia*	do.			2	1						
grayana	wild					0	1				
japonica	cultivated	1	0								
mahaleb	do.			2	0						
mume	do.					0	1				
persica	do.			1	0			2	0	3	0
spinosa	wild but with cultivated forms	1	0	1	15						
sp	——	2	0	2	5			2	0		

The demonstration of the existence of 2 forms confined to different host groups (*i.e.* cultivated and wild species) makes it advisable to modify the present concept of the species *Tranzschelia pruni-spinosae,* described in 1801 by Persoon (25), on the basis of a rust fungus he found on wild plum, *Prunus spinosa,* in Europe.

Although originally described from a wild host, the emended descriptions published by De Toni (30), Winter (32, p. 193), Arthur (3), and others, also include cultivated species as hosts of this fungus. The characters of the teliospores in these descriptions are clearly those of the *typica* type, and no description is given of the *discolor* type teliospore found on cultivated hosts.

Since the teliospores produced on wild species of *Prunus* in the United States are morphologically similar to those described by Persoon, it has been assumed that this form in the United States is identical with the European species. The *typica* type in the United States is associated with an aecial stage[5] on various native Ranunculaceae but, since there is now some confusion as to the aecial host of the *typica* type in Europe, the evidence that the form represented by the *typica* type in the United States is identical with Persoon's species rests solely on morphological characters of the teliospores. On the other hand, Kreig (19) proved in Europe that aeciospores of *Aecidium punctatum,* on *Anemone coronaria,* give rise to infections that produce the *discolor* type teliospores. This is the only type of teliospore found in the United States on the peach and allied species; and, when the experiments of Scott and Stout and the writer are recalled, it is clear that the successful infection of peach and other cultivated species was due to the presence of *A. punctatum* on cultivated *Anemone coronaria* in the United States. The fungus producing the *discolor* type teliospores on peach and other cultivated hosts in United States is, therefore, identical with the fungus producing the *discolor* type teliospores in Europe.

This type, however, has been demonstrated to be physiologically and morphologically distinct in the United States from the *typica* type. A name, therefore, should be applied to the *discolor* type to indicate this, for it is clearly not the fungus described by Persoon in 1801. Jacky (18) proposed the name *Puccinia discolor* Fckl., but, according to Winter (32, p. 193), Fuckel's fungus (14) is merely a variant of Persoon's species. The writer examined a portion of Fuckel's type material[6] and found that the teliospores (Fig. 2, I) were not the *discolor* type and Jacky's proposal is, therefore, not tenable.

To establish the *discolor* type as a new species would obscure the obvious close relationship existing between the 2 types. Furthermore, the specific name *pruni-spinosae* has been associated with the rust both on wild and cultivated species for at least 56 years. To end this long period of general

[5] Although *Aecidium hepaticatum* Schw. was originally described from *Hepatica acutiloba* DC. the writer considers the aecial stages on *Anemone quinquefolia* L. and *A. caroliniana* Walt. to be the same fungus, on the basis of the successful inoculation of various wild species of *Prunus* by several investigators in United States with aeciospores from these hosts.

[6] Fuckel, L. Fungi Rhenani exsiccati. No. 2121. 1867.

usage would only create confusion. This is especially true when it is realized that teliospores of the *discolor* type are produced so sporadically on some hosts[7] that routine collections made during most of the growing season could not be identified, as they would lack the spore form that is the criterion of the new species.

The writer, therefore, believes that the most logical procedure is to consider the 2 types as the varieties *typica* and *discolor*, respectively, of the variable species *Tranzschelia pruni-spinosae* (Pers.) Diet. A technical description embodying this disposition of the two types follows.

TRANZSCHELIA PRUNI-SPINOSAE

Aecidium punctatum Pers., Ann. Bot. Usteri 20: 135. 1796.
Puccinia Pruni-spinosae Pers., Syn. Fung. 226. 1801.
Puccinia Pruni DC., Fl. Fr. 2: 222. 1805.
Aecidium quadrifidum DC., in Poir. Encycl. Meth. Bot. 8: 235. 1808.
Puccinia Prunorum Link, in Willd. Sp. Pl. 6[2]: 82. 1825.
Uredo fusiformis Gachet, Act. Soc. Linn. Bordeaux 5: 232. 1832.
Aecidium hepaticatum Schw., Trans. Am. Phil. Soc. 11. 4: 293. 1832.
Uredo Pruni Cast., Obs. 1: 27. 1842.
Uromyces Prunorum Fckl., Jahrb. Ver. Nat. Nass. 15: 20. 1861.
Dicaeoma Prunorum Rabh., Fungi Eur. 990. 1866.
Puccinia discolor Fckl., Fungi Rhenani 2121. 1867.
Dicaeoma Pruni-spinosae Kuntze, Rev. Gen. Pl. 3: 470. 1898.
Aecidium dakotensis Griff., Bull. Torrey Club 29: 300. 1902.
Aecidium Aikeni Sydow, Ann. Myc. 1: 334. 1903.
Tranzschelia punctata Arth., Res. Sci. Cong. Bot. Vienne 340. 1906.
Tranzschelia pruni spinosae (Pers.) Diet., Ann. Myc. 20: 31. 1922.

Pycnia chiefly epiphyllous, evenly and remotely scattered, conspicuous, aecia hypophyllous, scattered, cupulate, dehiscent into few (often 4) widely spreading lobes; aeciospores globoid or oblong-globoid, 15–23 by 18–26 μ; wall cinnamon-brown, 1.5–2.5 μ thick, verrucose.

On Ranunculaceae: The species are listed under the two varieties.

Uredia hypophyllous, cinnamon-brown, pulverulent; urediospores oblong-clavate or oblong-fusiform, 15–23 μ by 28–42 μ, with capitate paraphyses intermixed; wall brownish-yellow above, paler below, 1–1.5 μ thick at sides, 5–9 μ above, smooth above, sharply echinulate below, the pores 3–5, equatorial. Telia hypophyllous, dark chestnut-brown, somewhat pulverulent; teliospores oblong or obovate-oblong, 18–27 by 30–39 μ, the two cells globoid or oblong-globoid and easily separable; the basal cell frequently smaller than the apical cell; wall chestnut brown, often paler in the basal cell, usually 1.5–2.5 μ thick but frequently thickened at top of apical cell, coarsely verrucose over both cells or the basal cell only sparsely so; pedicel colorless, readily detached from the spore.

On Amygdalaceae: The host species are listed under the 2 varieties.

Range: A cosmopolitan species, occurring throughout the world.

The rust fungus on the genus *Prunus* is a variable species and can be separated into two varieties according to the combination of aecial and telial hosts, as established by inoculation experiments, and by a difference in the morphology of the teliospores.

(1) **Tranzschelia pruni-spinosae typica** n. comb. (*T. pruni-spinosae forma typica* Fischer).

[7] For example, Scribner (29) stated in 1887 that he had never found teliospores on the peach and thought it was probable that they were not formed on this host. This is not the case, although they were found on only 20 of the 144 peach specimens examined by the writer.

Pycnia and aecia as above.

On Ranunculaceae: Anemone caroliniana Walt., *A. quinquefolia* L., *Hepatica acutiloba* DC. (*H. acuta* Britt.), *H. triloba* Chaix. (*H. hepatica* Karst.), *Ranunculus recurvatus* Poir., *Thalictrum dasycarpum* Fisch. and Lall. (*T. purpurascens* Arth., not L.), *T. dioicum* L., *T. polygamun* Muhl. (*T. cornuti* T. and G.) in North America; *Anemone ranunculoides* L. in Europe; and probably also in Asia on wild species.

Urediospores as above; teliospores with both cells of approximately the same size and color, the wall not noticeably thickened at top of the apical cell and uniformly coarsely verrucose over both cells.

On Amygdalaceae: Reported on various wild species of *Prunus* in North America, Europe and Asia, on the cultivated species *Prunus armeniaca* L., *P. cerasifera* var. *pissardii* Koehne, *P. domestica* L., *P. domestica* var. *insititia* Bailey, and *P. mume* S. & Z., in Europe and Asia.

Range: Throughout the Northern Hemisphere zone, wherever wild species of *Prunus* occur, and possibly extending into the Southern Hemisphere on species of *Prunus* growing in the Andes Mountains. Although morphologically similar forms of this variety occur in various hosts in many parts of the world, it is assumed only that they are identical. No inoculation experiments have been performed to confirm this assumption.

(2) **Tranzschelia pruni-spinosae discolor** n. comb. (*T. pruni-spinosae forma discolor* Fischer).

Pycnia and aecia as above.

On Ranunculaceae: Anemone coronaria L. in Europe as an indigenous species, elsewhere as a cultivated introduced plant. Reported definitely from the United States, Australia, and New Zealand.

Urediospores as above; telia scattered, generally produced sparsely except on *Prunus domestica* L., teliospores distinguishable from those of *T. pruni-spinosae typica* by the fact that the wall of the apical cell is uniformly and coarsely verrucose, while that of the basal cell is sparsely, if at all, verrucose. The apical cell is globoid with the wall frequently thickened at apex. The basal cell is oblong or obovate-oblong, generally narrowed toward base and lighter in color than the apical cell.

On Amygdalaceae: Prunus armeniaca, North America, Europe, Asia, and Australasia.—*P. avium,* Europe.—*P. besseyi,* North America.—*P. cerasifera,* South America.—*P. cerasifera* var. *divaricata* Ledeb., Europe.—*P. communis,* North America, Europe, Asia, Australasia.—*P. davidiana,* North America.—*P. domestica,* North America, Europe.—*P. hortulana,* North America.—*P. japonica,* South America.—*P. mahaleb,* Europe.—*P. mexicana,* North Ameria.—*P. mume,* North America.—*P. persica,* North America, South America, Europe, Asia, Africa and Australasia.—*P. spinosa,* Sôuth America, Europe.

Range: Throughout the world, wherever cultivated species of *Prunus* are grown. Found also on cultivated forms of some wild species.

The 3 species of *Thalictrum* and *Ranunculus recurvatus* Poir., are included, on the authority of Arthur, as aecial hosts of the *typica* variety in the

United States. The inclusion of *Anemone ranunculoides* as the European host of the same variety is based upon statements to this effect in the literature. The writer has not had an opportunity to examine aecial material on any of these hosts nor to use aeciospores from them in inoculation experiments.

The list of uredial and telial host plants for the 2 varieties is considered incomplete, but specimens of all those listed, except *Prunus cerasifera* var. *divaricata,* have been examined and the variety of rust fungus present on them identified.

The urediospores of both varieties show wide variation in size and shape, and no constant character was noted that would distinguish the urediospores of one variety from those of the other. Cristinzio (7), however, reported that he could distinguish between the urediospores produced on a number of cultivated hosts on the basis of size of spores and shape of the paraphyses. Since his cross-inoculation experiments showed no genetic variations that corresponded with the observed morphologic differences, it is evident that all the hosts (almond, apricot, plum and peach) were infected by the same variety. The differences he observed, therefore, are not differences between the urediospores of the varieties *typica* and *discolor* but are merely variations within the variety *discolor.*

Finally, it should be mentioned that *Eranthis hiemalis* (L.) Salisb. is not a host plant for either of the varieties although frequently considered as such in Europe. Tranzschel (31) has recently demonstrated that the fungus on this plant is the aecial stage of *Puccinia cerasi* (Bereng.) Cast., an entirely different rust from the one discussed in this paper. He has created a new genus *Leucotelium* to which he has transferred *Puccinia cerasi* (Bereng.) Cast. and *P. pruni-persicae* Hori. The teliospores of these rusts are two-celled, smooth-walled, hyaline spores quite different in appearance from the rough-walled, colored teliospores of *Tranzschelia.*

SUMMARY

The rust fungus commonly known as *Tranzschelia pruni-spinosae* (Pers.) Diet. is the cause of a disease of considerable economic importance on *Prunus* spp. throughout the world.

The disease is confined primarily to the leaves, but, under certain conditions, has been known to attack the fruit of the peach. Marked variations have been noted in the prevalence and importance of the disease.

In a study of the causes of these variations it was found that urediospores from cultivated hosts from various parts of the United States would infect peach leaves but would not produce infections on the leaves of wild species of *Prunus.* Conversely, urediospores from various wild species would not infect the leaves of the peach. Likewise, aeciospores from wild species of *Anemone* would not infect peach leaves but would infect leaves of wild species of *Prunus,* whereas aeciospores from a cultivated species of *Anemone* would infect peach leaves but would not infect those of wild species.

An examination of 389 herbarium specimens showed that the teliospores produced on the leaves of the cultivated species are morphologically different from those produced on the leaves of the wild species of *Prunus*.

These results, while they do not explain the variations noted in the prevalence and importance of the disease, do show that these variations are not due to occurrence of different species of the rust fungus on the cultivated species of *Prunus*, for the same type of teliospores was found on them throughout the world almost without exception.

The demonstration of the existence of 2 forms of rust fungus confined to different host groups (*i.e.* cultivated and wild species) in the genus *Prunus* necessitates a revision of the present concept of Persoon's original species *Tranzschelia pruni-spinosae*. This species is considered separable into 2 varieties according to the combination of aecial and telial hosts and differences in the morphology of the teliospores. The combination *Tranzschelia pruni-spinosae typica* is proposed for the variety found on wild species and *T. pruni-spinosae discolor* for the variety attacking the cultivated species. Technical descriptions of these new combinations are included in the text.

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A BACTERIOSIS OF DAHLIA, ERWINIA CYTOLYTICA

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INTRODUCTION

Very little thorough work has been done on the stem rots and blights of dahlia. In 1933 the late Dr. M. A. Howe observed a stem rot of dahlia then present in the New York Botanical Garden. Reference to this fact was made by M. T. Cook (2), and the fungus associated with the trouble was identified as *Sclerotinia libertiana* (Lib.) Mass. The disease in the Garden was confined to the Snowdrift and Shasta varieties.

As described by Cook, the first evidence of the disease is a water-soaked appearance of the stem, accompanied by wilting. Finally, the diseased parts die, and become black and dry. The central cavity of the stem is more or less filled with a luxuriant growth of mycelium, accompanied by the appearance of sclerotia. Beyond the identification of the fungus no experiments were undertaken, and its pathogenicity was not established.

Since then, the occurrence of the fungus on dahlia in other parts of the country has been recorded in the plant-disease surveys of the U. S. Department of Agriculture. It should be noted, however, that in the disease under

[1] Grateful acknowledgment is given by the writer to Dr. B. O. Dodge for assistance and advice and for the facilities of his laboratory.

discussion no such condition exists and no such fungus growth occurs in the decayed pith tissue.

In 1933, under the title: A New Disease of Dahlias, Thelma B. Post (5) of the Bureau of Plant Industry, U. S. Dept. of Agriculture, described a stem blight of dahlia with which a fungus was associated, identified as *Macrophomina phaseoli* (Maubl.) Ashby.

To quote the author: "Most of the stem tissue was blackened, but some water-soaked tissue was still evident. The pycnidia occurred in great numbers in the epidermis of the blackened areas. As the stem tissue dries the fibres separate easily and the ends are split and frayed. Most of the pith disappears leaving only a thin brittle remnant in which the sclerotia are embedded in such profusion that it had the appearance of a black crust." Cultures of this fungus from single pycnidiospores produced both sclerotia and pycnidia; or again, only sclerotia, previously identified as *Rhizoctonia bataticola* (Taub.) Butl., thus establishing the identity of the two stages. However, no plant inoculations were made to establish the pathogenicity of the fungus. Whether this organism is the same as that noted by Cook is an open question. However, in the disease under consideration no pycnidia developed on the epidermis, nor were there sclerotia in the pith tissue in specimens kept for a long time in a moist chamber.

In 1927[2] mention is made of a Fusarium wilt of dahlia occurring in the District of Columbia and in Missouri, but no studies were made of the disease at the time.

J. F. Adams (1), in 1927, makes repeated reference to the "Stunt" or "Dwarf" disease of dahlia, but was unable to attribute it to any single cause.

The first reference to bacteria pathogenic to dahlia is recorded by Hori (4), who described a wilt that attacks both stems and tubers, and the causal organism *Bacillus dahliae.* Hori's paper is not available, but Elliott (3) gives a brief description of the bacillus and the symptoms of the disease. According to Elliott, the organism belongs to the genus *Erwinia*, although the available data are insufficient to correlate it with the present organism, even though the symptoms are similar.

In 1922 Wolf (6) isolated from the xylem tissue of dahlia a bacterium that he considered the same as *Phytomonas solanacearum* (Erw. Smith) Bergey *et al.*, ordinarily known as the cause of brown rot of Solanaceae. Wolf made no inoculations; hence, we must reserve opinion as to its pathogenicity for dahlia. Realizing the importance of Wolf's work, however, we attempted to find this organism in our own material, but were unsuccessful and, therefore, concluded that the bacterial trouble under consideration was of different origin. Since then, the occurrence of a bacterial wilt of dahlia has been reported, attributable to *Phytomonas solanacearum,* apparently on the assumption that it was the same as that reported by Wolf.

[2] Martin, G. H. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1926. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. Sup. 55. 1927. [Mimeographed.] (See p. 365.)

BACTERIOSIS OF DAHLIA

In late August, 1936, attention was called to a stem rot of dahlia in the Garden. This disease becomes externally recognizable through a blackening and softening of the stem. Stems cut open lengthwise show a moist and advanced decay of the layer of pith lining the hollow stem, whence it extends into the cortex, which becomes blackened. The decaying stems swarm with bacteria and give off a foul odor not unlike that of mice.

To isolate the bacteria, an infusion of the decayed tissue was made in sterile water from which beef-peptone-agar plates were poured, which, in high dilutions, gave numerous colonies, mostly of a prevailing type, and frequently also, a few colonies of a larger spore-forming bacillus, apparently not a pathogen.

The former prevailing organism gave colonies (Cult. Dal. 2. Table 1) of a distinctive appearance, which were subsequently easily recognized in other isolations. In two days at 25°–30° C., they average about 2 mm. in diameter, are circular, convex, watery, glistening, pale grayish, and translucent. By transmitted light they are strongly refracting, pale drab to light brownish-yellow, nearly or quite amorphous, with sharply defined, entire borders. The bacteria are actively motile, short rods averaging 0.7×1.5–$4\ \mu$, occurring singly and in pairs. The flagella are peritrichous, thus definitely placing it in the genus *Erwinia*.

To determine whether this organism (Dal. 2), which we shall designate as the original isolation, is pathogenic to dahlia, sections of healthy stems were washed clean in running water, then placed for 5 minutes in 1:1000 $HgCl_2$ solution, the latter removed by rinsing with sterile water. The stems were then placed in sterile Petri dishes and inoculated by puncture with a pure culture of the organism in question. Drying out was prevented by placing the dishes under a bell glass. Checks were made by punctures with a sterile needle.

Generally, within 2 days, a decided decay had set in from the points of inoculation, which continued to extend into the pith. Bacteria were abundant. To compare these with those used as inoculum, plate cultures were grown upon beef-peptone agar. In all cases there developed numerous colonies of the type already described. Transfers were made from these to agar and to different media to establish the identity of the separated organism with the original isolation. These inoculations were repeated in sufficient number to confirm previous tests regarding the pathogenicity of the organism.

The occasional presence of the aforementioned sporulating *Bacillus*, which seemed to be the same species throughout, raised the question whether it might not also be pathogenic or at least contribute to the decay. Accordingly, stems were inoculated with a pure culture of the latter spore-forming organism. No infection resulted in any case, while stems inoculated simultaneously with the original isolation (Dal. 2) showed advanced decay within 3 days. The same decay resulted from inoculations with a mixed suspension

of the two organisms showing at least that the presence of the sporulating *Bacillus* did not inhibit the infection of the other.

In bacteriosis of the dahlia, the decay generally starts at the crown, indicating that the original infection comes from the soil, although this is not always accompanied by any marked decay of the tuber. The bacteria at first make slow progress in the cortex, and the more rapid growth in the pith tissue is due partly to its parenchymatous character and perhaps also to the presence of air in the hollow stem. When, however, the pith becomes thoroughly decayed, soft, and watery, the bacteria rapidly invade the outer layers, when bacteria are present in great numbers. As soon as the outer ring of woody tissue becomes involved, it results in complete softening and disintegration, the coherence of the woody fibres is destroyed because of the intracellular dissolution.

Whenever tuber decay accompanies that of the stem, we would expect to find the same organism in the tuber. That the same is true is shown by our isolates in series IX, table 1.

TABLE 1.—*Results obtained from artificial inoculation of dahlia stems with Bacillus, Dal. 2, from cultures grown in colony plates and inoculated stems*

Ser.	Cult.	Inoculant	Source of culture	Group number
I	Dal. 2	Decayed stem		5010.32120.1222.
I	Dal. 2–5	Dal. 2		2201.00.211.22.100.0
III	Dal. 2–1	Dal. 2	Colony Plate Inoc. Stem	The other cultures in the table have the same group numbers.
III	Dal. 24–2	Decayed pith	do	
V	Dal. 2–A–1	Dal. 2	do	
VI	Dal. 20–2	Dal. 2	do	Group numbers according to descriptive chart.
VII	Dal. 18–2	Dal. 2		
VIII	Dal. 23–2	Dal. 2	do	Soc. Am. Bacteriologists, Dec. 30, 1929.
VIII	Dal. 19–1	Mixed cult Dal. 2 and Fusarium	do do	
IX	Dal. 29–1		Decayed tuber	
X	Dal. 2		do	

Slices of tubers also were inoculated with a pure culture of the original isolate (Dal. 2), with the result that decay followed within 3 days.

Table 1 shows the main cultural and physiological characteristics of the organisms isolated from naturally decayed dahlia stems and tubers, together with those isolated from inoculated material.

Series I is the original isolation from a decayed stem, Series IX the same isolation from a decayed tuber. Both organisms are the same. Series X is an isolation from tuber sections inoculated with the original isolate which showed advanced decay within 3 days; also the same. The other isolate, series III, V, VI, VII and VIII, were from decayed stems, inoculated with the original isolate.

THE ORGANISM

The present organism does not agree with any of the recorded species in the genus *Erwinia,* hence, it is regarded as new and the name **Erwinia cytolytica** is proposed. The following is a description of its cultural and physiological characteristics.

Morphology. From cultures grown on beef-peptone agar, filmed, fixed in the flame, and stained with carbol-fuchsin, the vegetative cells average 0.6–0.7 μ in diameter by 2.5–3.5 μ in length, with occasionally longer forms up to 5.0 μ. Fixed with Loffler's mordant and stained with carbol fuchsin, they are broader, up to 1.0 μ, indicating the presence of a capsule that also could be demonstrated by the usual methods of staining. They occur singly and in pairs, and are gram-negative. Stained by the Van Ermingen method, the flagella are extremely delicate and arise from any part of the body of the cell. They are, therefore, peritrichous.

Spores. Absent. Broth, inoculated with a fresh culture, gave no growth when heated in water bath for 15 minutes at 75° C., while nonheated check tubes gave good growth.

Agar Colonies. On beef-peptone agar pH 7.2, 2 days at 25°–30°, 2–3 mm. diameter, round, convex, moist, glistening, grayish white, watery, translucent; by transmitted light, low magnification, light brownish yellow, amorphous; borders distinct, entire, undulate.

Agar Slant. Same medium as above. Growth thin, nearly white, translucent, smooth, moist, glistening, butyrous. Medium not discolored. The growth is more profuse on the same medium containing dextrose.

Beef-peptone Broth. pH 7.2. A uniform turbidity, no surface growth, floculent sediment.

Gelatin Stab. Medium slowly liquefied. At first, a crateriform depression, becoming infundibuliform in 7 days at 20° C.

Potato. Growth light brown, moist, glistening, smooth or widely contoured, medium not distinctly darkened.

Litmus Milk. Coagulated in 5–7 days at 25°–30° C., slightly acid; not digested; litmus reduced.

PHYSIOLOGICAL CHARACTERS

Aerobic and facultative anaerobic. In fermentation tubes of dextrose and sucrose broths, growth in closed arm; nitrates reduced to nitrites, indol-negative; H_2S not formed.

Starch hydrolyzed; acetylmethyl carbinol, a slight reaction over night. Ammonia production in beef-peptone broth, negative, only a slight increase in alkalinity.

Fermi's solution, good growth; Cohn's solution, no growth. No invertase in sucrose broth. In synthetic media containing mineral salts and ammonium phosphate as a source of nitrogen, acid produced, without gas, from dextrose, lactose, sucrose, raffinose, mannite, salicin, isodulcite. No acid from levulose, arabinose, xylose, glycerose, inulin. In synthetic media, as before, with dextrose as a source of carbon, good growth with asparagin, peptone, and ammonia as a source of nitrogen. No growth from KNO_3 as a source of nitrogen.

Pectinase Production. To test the production of this enzyme a fresh broth culture of the organism was filtered through a sterilized thimble of unglazed porcelain into a sterile flask. To the filtrate 0.5 per cent of thymol was added, and then distributed into sterile test-tubes. All these tubes remained sterile.

Thin slabs, under asceptic conditions were cut from a raw dahlia tuber, and transferred to tubes containing the sterile filtrate. A few of these showed contamination; the others remained sterile and clear.

Slabs in the latter tubes began to show in a few days signs of softening and dissolution, which increased until, on shaking, the tissue fell apart into a soft mushy mass.

Other tubes similarly treated were heated for 10 minutes in a boiling water bath. The slabs in these tubes remained intact. No effort was made to precipitate the enzyme.

Temperature relations. Optimum temperature 28°–30° C. Grows at 37° C. Slow growth at 20°, no growth at 8°–10° C.

Relations of Media. Approximately equal growths in beef-peptone broth at pH 6.8 to 7.3. Feeble growth at pH 5.0. No growth at 4.4.

SUMMARY

A stem rot of dahlia, which also affects the tubers, produced by a bacterium considered new and named *Erwinia cytolytica.* The organism has been isolated from decayed stems and tubers and its characteristics determined. Inoculations of pure cultures into dahlia stems and tubers gave the characteristic soft rot and from which the same organism was recovered.

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A ROOT ROT OF PEAS CAUSED BY FUSARIUM COERULEUM

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(Accepted for publication February 17, 1938)

The pea, *Pisum sativum* L., root-rot complex is composed of a number of different soil-inhabiting fungi that cause symptoms so interrelated that they cannot be identified with certainty by a macroscopic examination. Several of these fungi, widely separated in relationship, may occur in the same field, and more than one have been found to parasitize the same plant. The various organisms and the diseases they cause have been subjects of study by different investigators, both in the United States and in foreign countries (3), for a number of years, with the result that new parasites have been added to the list from time to time. The literature on pea diseases and in particular on the root rots is so extensive that no attempt will be made to review all or any considerable part of it.

In 1936, Geach (4) published an article on the root rot of grey peas that he thought was caused probably by a strain of *Aphanomyces euteiches* Drechsler, and, a few years earlier, Brown and Evans (1) described two diseases of peas new to Arizona, one of which, a species of the genus *Fusarium*, had never been associated previously with any disease of the crop. Other species of *Fusarium* have been isolated from peas and some of them proved to cause extensive damage to the crop. Jones (8) in 1923, published the results of his investigations on the stem and root rot of peas in which he showed that a new variety of *F. martii* that he described as *pisi* caused extensive losses in various parts of the United States. While species of *Fusarium* are known to cause extensive root-rot losses, the greatest damage is believed to be caused by members of the Phycomycetes (2, 4, 5, 6, 7, 9), among which may be mentioned species of *Pythium* and *Aphanomyces*. Their known distribution over the world is probably more extensive than that of any other root-rot fungus.

From several field surveys of pea diseases in the United States, the writer isolated with considerable frequency a species of *Fusarium* among other organisms that proved to be different from any heretofore described as a cause of pea root rot. Since it causes symptoms somewhat resembling those caused by *Fusarium martii* App. and Wr. var. *pisi* Jones, it probably was long overlooked as a possible parasite. While culturally and morphologically it was different from *Fusarium martii pisi,* there was sufficient resemblance from a casual examination to be mistaken for it. However, a more critical study of the organism showed that it was definitely a different species of *Fusarium.* In view of this fact experiments were undertaken to determine if it caused root rot of peas or was merely another saprophytic invader of dead plant tissue. This article discusses the results of the investigations with the organism and the disease it causes.

ECONOMIC IMPORTANCE AND DISTRIBUTION

There is no way of estimating, even approximately, the losses caused by this disease. It is induced by only one of several organisms that cause root rots of peas and occurs along with others in the field. The causative organism is one of those fungi that may or may not kill the plant but that retard its growth and consequently reduce the yield. The degree of resultant damage naturally depends on the age of the plant when attacked by the parasite and the kind of weather that follows.

To determine its distribution is not so difficult, although it is not entirely known. The causal organism has been isolated from the roots of pea plants in Maryland, Virginia, Colorado, Idaho, and Maine, and its distribution may be coextensive with that of the pea. Like other root-rot organisms, it is most abundant in those fields that have been long under cultivation and especially those fields planted frequently to peas.

SYMPTOMATOLOGY

Symptoms of a root rot under field conditions where there is competition with different pathogenic organisms are difficult to describe. The clearest

description can be made from plants infected in sterilized and, later, inoculated soil.

Under these conditions the causal fungus is particularly aggressive. Many of the seeds are rotted before they germinate and many of those that germinate are destroyed before the plant emerges through the soil, or soon thereafter, by decay the tap root, thereby leaving only a stub at the region of the attachment of the root to the seed. If, however, the plant escapes early decay, the first symptoms appear on the tap root in the region of its attachment to the seed and consist of small, black spots and streaks that are first observed about 3 weeks after planting the seed. As the plant grows, the infection areas extend up, down, and around the tap root (Fig. 1, A) to the soil line, involving most of the cortex. In the later stages of the disease some of the lateral roots (Fig. 1, B) become infected. The plant is rarely killed if it attains a height of 6 or 8 inches before it is attacked by the fungus.

Many of the symptoms of this disease are characteristic of the root rot of peas shown by Jones (9) to be caused by *Fusarium martii* var. *pisi*. The only sure way to determine which organism is causing the disease under field conditions is to make isolations and identify the fungus.

ETIOLOGY OF THE DISEASE

The causal organism is a species of *Fusarium* belonging to the section *Martiella*. While the spore sizes of the different isolates studied do not entirely agree with those given by Wollenweber and Reinking (10) for *Fusarium coeruleum* (Lib.) Sacc., the fungus agrees culturally and morphologically more nearly with that species than with any other. There is considerable variation in spore sizes, which average larger than those generally given for the species. The writer, however, believes that if it is not identical with *F. coeruleum* there is no more than a variety difference.

PATHOGENICITY

Many different fungi, among them several species of *Fusarium*, have been isolated from the underground parts of pea plants. Not all of them were parasitic and most of them were eliminated by inoculation tests or were identified as species previously proved to be root rot producing fungi. Those that showed any promise of being parasites were tested by additional inoculations. Among the organisms tested in an exploratory way was *F. coeruleum,* which proved to be a vigorous parasite; in fact, even more so than *F. martii* var. *pisi*. In view of these facts controlled inoculation experiments were carried out with different isolates. All the isolates were obtained from the roots of peas except one, which was furnished the writer by Freeman Weiss who isolated it from potatoes held for some time in storage.

A few preliminary experiments were conducted in the greenhouse to determine the parasitism of the causal organism in sand cultures. The sand was sterilized by heating 5 hours at about 10 pounds' pressure. Oats on

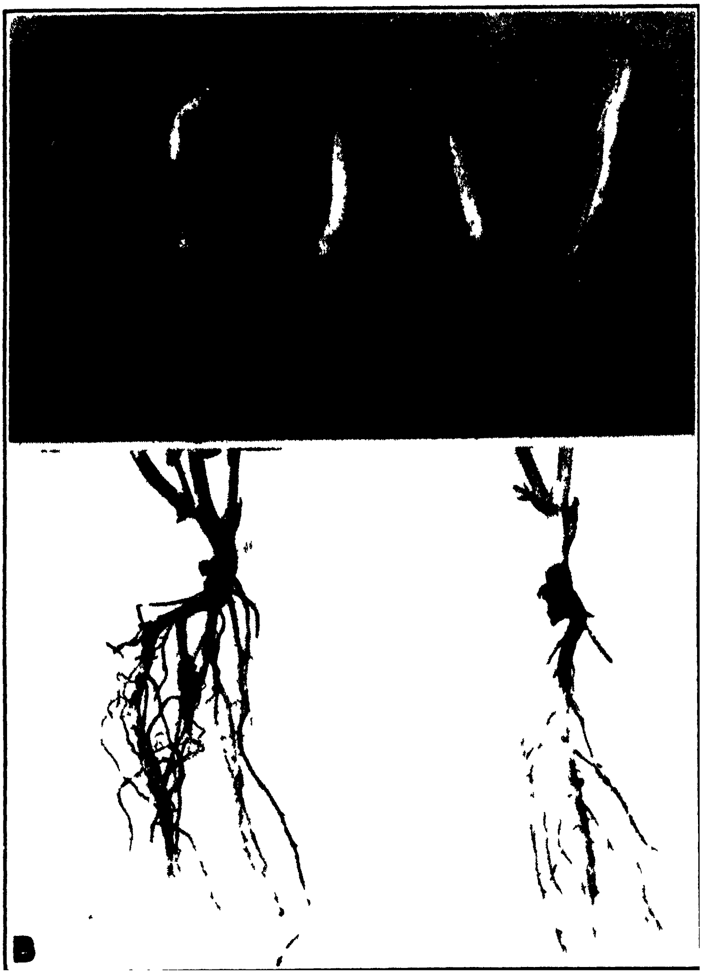

FIG. 1. Pea root rot caused by *Fusarium coeruleum*. A. Various types and degrees of infection of seedlings prior to or at time of emergence from soil. In all cases, cortex of tap root was destroyed. The pathogen already had attacked the stem in some cases and not in others. B. Two plants attacked when about 4 weeks old. Causal organism has extended up to the stem almost to surface of soil and downward onto tap root and rootlets.

which *Fusarium coeruleum* had grown for about 2 weeks were worked into the sand and 2 days later pea seed (Little Marvel and Harrison Glory varieties) was planted. Seven days later a few plants came up, but a careful examination later showed that most of the seed or plants had been destroyed before they emerged through the sand. Duplicate preliminary experiments gave similar results. These experiments were supplemented by the use of sterilized soil. A culture of the organism was grown on oats and stirred into the soil and 2 days later 400 seeds each of Little Marvel and Harrison Glory were planted. One hundred seeds of each variety were planted, as controls, on the noninoculated soil. In these experiments most of the plants came up and grew fairly well. At the end of 4 weeks they had attained a height of

a foot or more, when they were removed from the soil, washed, and examined for root-rot lesions. At that time 100 per cent of the plants were diseased, and most of them showed a conspicuous blackening of the cortex on the lower part of the stem and on the tap root and some laterals. None of the controls were diseased. They were longer and better plants than those in infested soil, showing that, although the vines looked healthy, growth was retarded. A duplicate planting was made on the same bed of soil immediately on removal of the first crop without the addition of any more inoculum. The second experiment gave almost identical results. All the plants became infected, but not so early as when the seed was sown 2 days after mixing the inoculum into the soil. It is interesting to note in connection with these experiments that, although the cortex was badly diseased, the foliage showed little evidence of any root trouble. Because of the frequent watering under greenhouse conditions, the plants evidently withstood the disease much better than they would had they been grown under normal field conditions.

VARIETY TEST

Twenty-four varieties were selected for testing in soil that had been steam-sterilized 7 hours on 3 consecutive days at about 10 pounds' pressure. The soil was spread on a bench in the greenhouse and inoculated with oat cultures, as described previously. Two days after the soil was inoculated, one half of it was planted, using 25 seeds of each variety. The Little Marvel, previously shown to be susceptible, was used as a susceptible control on noninoculated soil. The second planting was made 7 days later. Only a small percentage of the seed of the first planting came up and the resultant seedlings were badly diseased and died soon thereafter. Most of them were killed before they emerged from the soil. The second planting did much better. More of the seed came up and the young plants grew well. In about 18 days after planting the cortex of the lower stem and of the tap-root turned black. The plants were removed from the soil and examined for root rot at the end of 30 days with the results shown in table 1.

An examination of table 1 shows that none of the varieties are immune from the disease; in fact, it is doubtful if any of them could be classed as tolerant. The probabilities are that those varieties in which some plants were only slightly or moderately diseased, escaped infection for a time and, consequently, did not show the same degree of injury when the experiment was terminated. No resistant varieties were discovered by these experiments. It is, however, interesting to note that, under the conditions of these tests, the parasite did not destroy the germinating seed and seedlings so actively after it had been in the soil for a week or more as it did when first introduced.

The pigeon pea, *Cajanus indicus,* was almost completely immune.

TEMPERATURE RELATIONSHIP

Several attempts were made to fix the cardinal temperatures for the growth of the organism in conjunction with the host by means of tempera-

TABLE 1.—*Susceptibility of 24 varieties of peas to the pea root-rot organism, Fusarium coeruleum.* (*25 seeds planted*)

Variety	Number of plants to emerge	Degree of infection
Pisum sativum		
Alaska	15	3 moderate, 12 severe
Alderman	12	12 severe
Asgrow Alaska	14	14 "
Blue Bell	11	11 "
Canners Perfection	18	18 "
Capucijner	22	22 "
Daisy (Dwarf Telephone)	14	14 "
Horsford	16	6 slight, 10 moderate
Hundredfold	15	15 severe
Large Podded Alaska	16	1 slight, 15 severe
Laxtons Progress	12	12 severe
Little Marvel	7	7 "
Onward	13	13 "
Perfection	10	10 "
Pioneer	21	21 "
Potlatch	3	3 "
Stratagem	12	12 "
Surprise	8	8 "
Telephone	12	5 slight, 4 moderate, 3 severe
Thomas Laxton	17	17 severe
World Record	20	20 "
Pisum sativum arvense		
Austrian Winter	24	24 severe
Maple	25	25 "
Victoria	20	20 "

ture-controlled soil tanks. Briefly, the method consisted in the use of sterilized soil into which was mixed oat cultures of the fungus. Seed of the varieties to be tested was planted a day or two later. The temperatures covered a range from 10° to 40° C. at 5° intervals. Many of the seeds were destroyed as soon as germination started and only a few emerged from the soil. Almost no plant growth occurred at the low and high temperatures. The soil was prepared to contain 35 per cent of its water-holding capacity and maintained at that amount by adding water daily to bring it to weight. The soil contained sufficient moisture for seed germination and plant growth. The failure of the above described method suggested the possibility of germinating the seed in pure sand and then transplanting to the soil tanks after a copious root system had been developed. The plants did not survive transplanting at the extreme temperatures and at intermediate temperatures recovery was very slow. Lacking the vigor of thriving plants they soon succumbed to the attacks of the fungus. An analysis of the data obtained by these experiments allows only one conclusion to be drawn, *i.e.*, the causal organism has a temperature range of parasitism that corresponds to the temperatures at which the host will grow.

SUMMARY

A root rot caused by *Fusarium coeruleum,* not previously recognized as a parasite of peas, has been discussed. The causal organism is shown to be

a vigorous parasite, and widely distributed in the United States.

Twenty-four varieties of peas were planted in artificially infested soil and no resistance or tolerance was shown by any of them. Pigeon pea, *Cajanus indicus*, is immune.

The temperature range of the pathogen corresponds to that of the temperature permitting growth of the host.

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EYE-SPOT DISEASE OF NAPIER GRASS

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(Accepted for publication February 10, 1938)

In 1935, Leukel and Camp[1] reported that the yields of Napier grass, *Pennisetum purpureum* Schum., in their fertility plots at Gainesville, Florida, were severely affected by a disease, and that selections were being made for resistance from the few plants remaining healthy. Later, Stokes and Ritchey[2] reported that the disease was caused by *Helminthosporium ocellum* Faris, and that resistant strains of the grass had been found, the organism being identified by the writer. In 1928, *H. ocellum* was reported by Faris[3] as causing eye spot of sugar cane in Cuba, Puerto Rico, and Santo Domingo. In 1934, Bourne[4] also proved that this fungus was the organism causing eye

[1] Leukel, W. A., and J. P. Camp. Fertilization of pasture and forage grasses. Fla. Agr. Exp. Station Ann. Rpt. 1934–35: 41. [1935?].

[2] Stokes, W. E., and G. E. Ritchey. Crop Adaptation Studies. Fla. Agr. Exp. Sta. Ann. Rpt. 1935–36: 42. [1936?].

[3] Faris, James A. Three Helminthosporium diseases of sugar cane. Phytopath. 18: 753–774. 1928.

[4] Bourne, B. A. Studies on the ring spot disease of sugar cane. Fla. Agr. Exp. Sta. Bull. 267. 1934.

spot of sugar cane in Florida, and that when accompanied by such secondary organisms as *Phyllosticta sorghina, Nigrospora* sp. and *Leptosphaeria sacchari,* a typical ring-spot disease is produced. No typical ring spots were observed on the Napier grass at Gainesville, although sugar cane, affected with ring spot, was growing in the immediate vicinity; consequently the disease affecting Napier grass will be referred to only as an eye spot.

Since the symptoms of the disease as it occurs on sugar cane, and the morphology and physiology of the causal organism have been described by

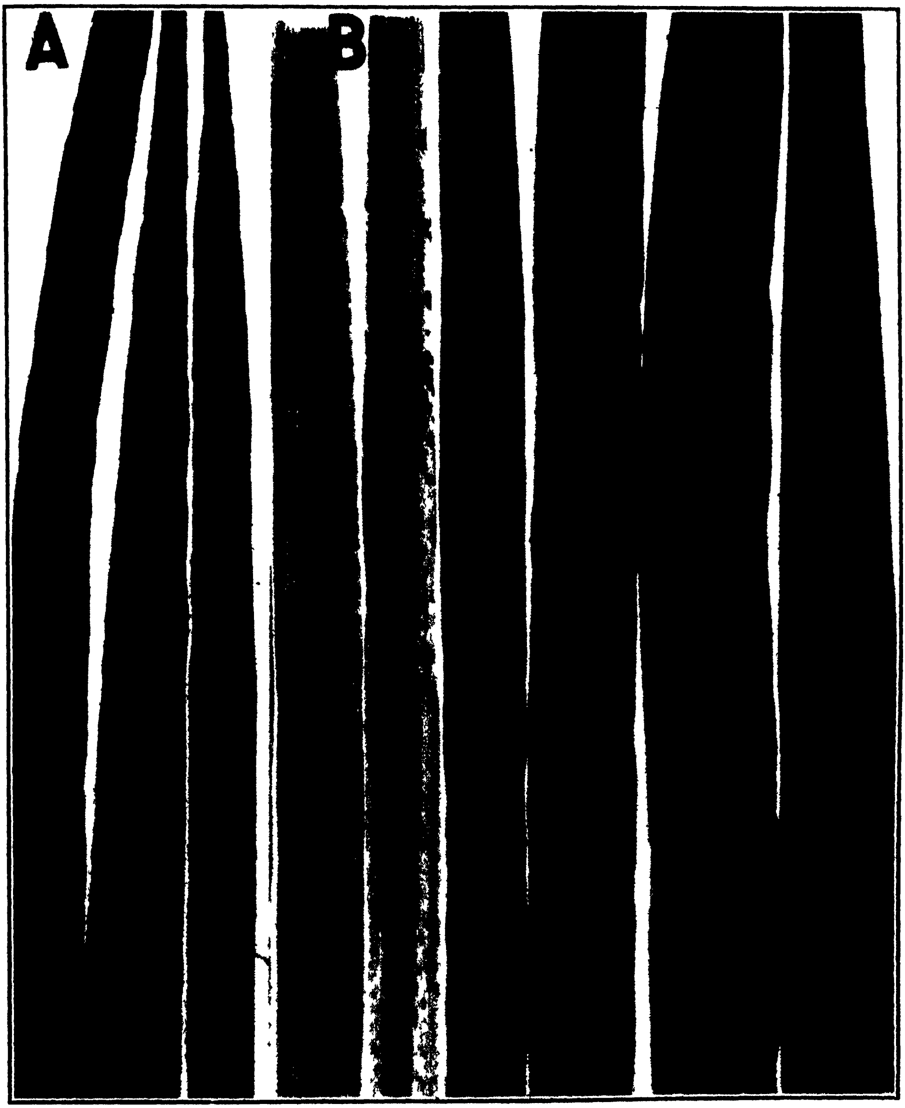

FIG. 1. Typical eye spots on leaves of Napier grass. A. Artificially inoculated in the greenhouse B. Left, naturally infected leaves, showing different stages in the development of eye spots; right, leaf from a resistant plant.

Bourne,[5] this paper deals only with a description of the disease as it occurs on Napier grass, the proof that the causal organism is *Helminthosporium ocellum* and a report of the results of inoculation tests conducted in the greenhouse.

FIG. 2. A. Portion of a Napier grass leaf showing eye spots enlarged. × B. Left, typical spots on leaf sheaths; right, stalk from a resistant plant.

DESCRIPTION OF THE DISEASE

The young spots on the leaves first appear as small, reddish-brown, somewhat oval flecks. On aging they enlarge, the center becomes lighter brown, and the margins a deeper red, until, finally, the centers are of a dirty straw

[5] See footnote 4.

color and the margins a Bordeaux red. The red color predominates in the older spots and the leaves usually assume a reddish cast. By transmitted light, the older spots (Fig. 2, A) usually show a dark fleck in the center surrounded by one or two alternating light and dark areas. The older spots vary in size from 1.5–3 mm. wide by 2–5 mm. long, the greatest dimension being always in the direction of the long axis of the leaf. The spots (Fig. 1, A) tend to remain somewhat oval and regular in outline, except where two or more coalesce and the margins that come in contact disappear, leaving a blotch of irregular outline. The spots on the leaves of some strains of Napier grass develop into elongated streaks similar to those on sugar cane leaves. In cases of heavy infection the leaves wither and die prematurely, the basal leaves first, and drop to the ground.

On very susceptible strains of this grass, the leaf sheaths and stems also

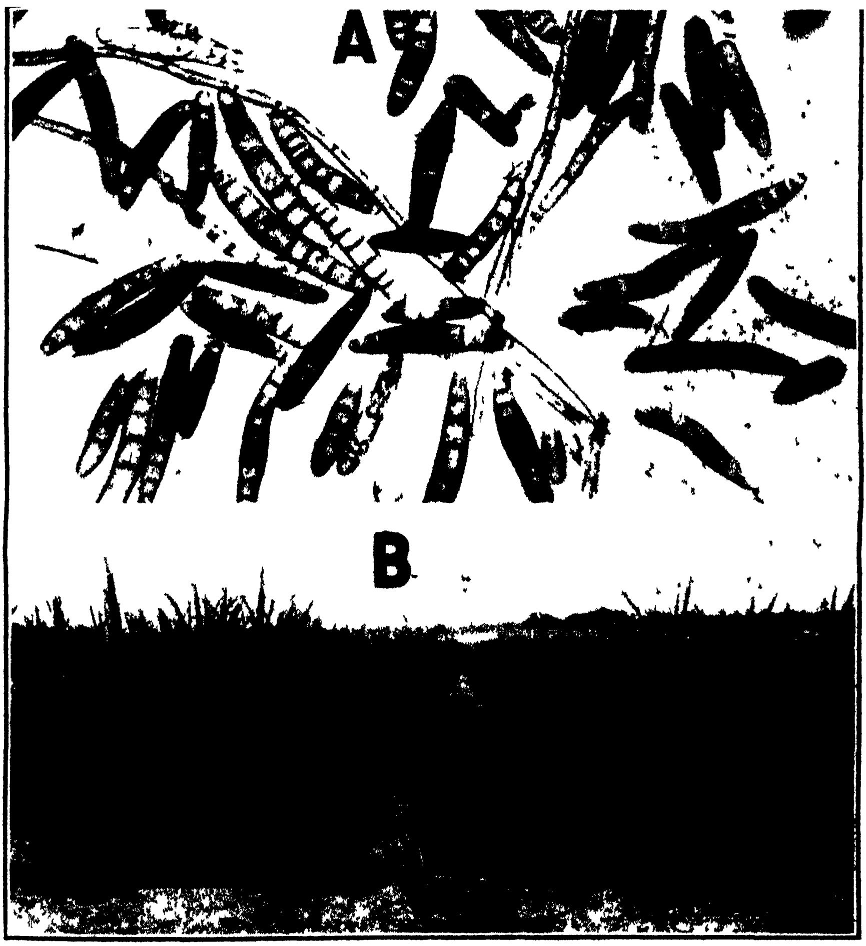

FIG. 3. A. Typical spores of *Helminthosporium ocellum*. ×400. B. View of Napier grass plots showing plants of resistant strains in two outside rows and plants of susceptible strains in center row. Selections made by G. E. Ritchey.

may become heavily infected. The young spots on these parts are similar to those on the leaves, except that a brown rather than red color predominates in all stages in the development of the spots. As the spots become older the contrast in color of the center and margin is much less conspicuous than on the leaves; the entire spot tends to remain brown, the color becoming diluted toward the edges of the spot. The spots (Fig. 2, B) do not become so large as those on the leaves and tend to coalesce and form large, brown, sunken areas.

INOCULATIONS

Young Napier grass plants from root cuttings and seed of resistant and susceptible plants, field-selected by G. E. Ritchey[6] (Fig. 3, B), were grown in the greenhouse and inoculated with a water suspension of spores from pure cultures of *Helminthosporium ocellum* growing on potato-dextrose agar. None of the plants from stock that was immune from the disease in the field became infected in the greenhouse, but all of those originating from susceptible stock developed eye spot in varying degrees, except the noninoculated checks; thus demonstrating that selection of strains of Napier grass naturally immune from or susceptible to the disease can be done effectively in the greenhouse.

A few, small, water-soaked flecks appeared on some of the leaves of the susceptible plants 12 hours after inoculation, but the majority did not appear until after 24 hours. The fresh spots that developed from the flecks closely resembled those that appear on leaves in the field, but as they became older they were surrounded by a distinct halo (Fig. 1, A), which is not so evident on naturally infected leaves. Although *Helminthosporium ocellum* was the only organism isolated from eye spots of Napier grass leaves in both the field and greenhouse, the complete range of morphological characteristics of the disease, as it occurs in the field, was not reproduced by inoculation in the greenhouse, probably because of differences in the environmental conditions of field and greenhouse.

TABLE 1.—*Comparison of spore measurements and number of septa of Helminthosporium ocellum on sugar cane and Napier grass*

Author and host	Length	Average	Width	Average	Number of septa	Average
Faris, Cuba—on sugar cane	29–84 μ	68.9 μ	9–21 μ	12.7 μ	3–10	6.7
Bourne, Florida—on sugar cane	24.1–92.5 μ	58.7 μ	11.1–16.7 μ	14.0 μ	3–9	6.7
Voorhees, Florida—on Napier grass	28–90 μ	56.4 μ	8–14 μ	11.1 μ	3–9	6.5

[6] Associate Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, and the Florida Agricultural Experiment Station.

THE CAUSAL ORGANISM

The fungus isolated from eye spot of naturally infected leaves of Napier grass was grown in culture and its morphological characters studied. When 200 spores of the fungus were measured, the length, width, and number of septa agreed very closely with the dimensions of *Helminthosporium ocellum* as reported by Faris[7] and Bourne[8] (Table 1). Other characters, including color and type of mycelial growth in culture, color of spores and conodiophores, marked bulbous base of conidiophores, and slight curvature of mature spores (Fig. 3, A), also agree with descriptions given for *Helminthosporium ocellum* and it is concluded that the fungus causing eye spot of Napier grass is the same as that causing the eye or ring spot of sugar cane.

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PHYTOPATHOLOGICAL NOTES

Bacterial Wilt of Sweet Corn in Mexico.—Bacterial wilt of sweet corn has been reported from time to time from most of the southern United States.[1] The disease occurs in the Carolinas, Georgia, Tennessee, Arkansas, and Oklahoma and has been found also in the southern border States of Mississippi, Texas, and New Mexico. The corn flea beetle, *Chaetocnema pulicaria,* has been "reported from most of the United States except the Northwest." "This species was collected by airplane during nearly every month throughout the year at Tallulah" [La.].[2]

Outside of the United States, bacterial wilt has been reported from Puerto Rico and Italy.

While on a vacation trip in Mexico, in December, 1937, green corn was found growing in the lower, more tropical sections in the vicinity of Orizaba, Oaxaca, and Jalapa. At Orizaba and Jalapa, what appeared to be bacterial-wilt lesions were found on the leaves. These lesions were local and typical of the type of lesion commonly occurring on more or less resistant field corn. On returning to Washington, D. C., sections of some of these lesions were examined under the microscope, and bacteria streamed out from the cut ends of fibrovascular bundles. Yellow bacterial colonies developed on plates poured from the lesions on the specimens collected at Orizaba. Pure cultures transferred from these colonies produced typical wilt symptoms on young sweet-corn plants in the greenhouse at Arlington Farm, Virginia. Pure cultures of a yellow organism reisolated from these young sweet-corn plants have again produced typical wilt symptoms on young sweet-corn plants. In sev-

[7] See footnote 3.

[8] See footnote 4.

[1] Rand, F. V., and Cash, L. C. Bacterial Wilt of Corn. Tech. Bul. 362, U. S. D. A., revised, 1937.

[2] Poos, F. W., and Elliott, C. Certain Insect Vectors of *Aplanobacter stewarti.* Jour. Agr. Res. [U. S.] 52: 585–608. 1936.

eral cases the cultures were sufficiently virulent to kill the inoculated plants. The growth of these pure, yellow cultures on beef peptone agar and beef peptone broth is typical of *Aplanobacter stewarti* (E.F.S.) McC. and, since the lesions produced on corn are typical of bacterial wilt, there can be no question of the occurrence of this disease in Mexico. While corn appears to be grown in all sections of Mexico, even up to 9,000 feet, or higher, on the mountain slopes, in December all of the corn on the plateaus and mountain slopes was mature and much of it harvested. Consequently, it was not possible to make observations of the abundance and distribution of the disease in Mexico at that time. All of the teosinte plants found were also mature and dry and no information was obtained on the occurrence of the disease on this natural host, which is native in this area.

On the green corn plants infected with wilt in the vicinity of Orizaba, insect feeding injuries similar to those caused by *Chaetocnema pulicaria* were observed on the leaves and some of the smaller lesions appeared to be developing from these feeding injuries. A few small flea beetles, which appeared to be *Chaetocnema pulicaria,* were collected from these corn plants and were submitted for identification to H. S. Barber, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, who found the single included male, from Orizaba, to agree in all external details as well as in the peculiar shape of the aedeagus with those of *C. pulicaria* from the eastern United States and who believed the several female specimens from Orizaba and Oaxaca to belong also to that species. Apparently, therefore, *C. pulicaria* occurs in the vicinity of Orizaba and Oaxaca, Mexico, and probably serves as a vector for the bacterial-wilt organism there, as well as in the United States. No isolations were made from these beetles collected in Mexico.—CHARLOTTE ELLIOTT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Observations on the dissemination of fungi by ants.[1]—Bailey[2] in 1920 presented a comprehensive discussion of "Some relations between ants and fungi" in which he showed that the infrabuccal pellets of many ants often contain large quantities of fungus spores and mycelium. He states that "It is the general tendency among ants to take spores, fragments of mycelia, and bits of decaying plant tissue into their infrabuccal pockets. In this sac the fungi become mixed with food residues and mineral and vegetable detritus, forming a pellet which is subsequently voided upon any convenient surface." The composition of the infrabuccal pellet suggests that it is a "culture or potential source of infection." He pictured the contents of several pellets in which could be recognized the spores of many fungi belonging to genera that include important plant pathogens. His studies were made on specimens preserved in alcohol, and the viability of spores in fresh

[1] Paper Number 1578 of the scientific journal series, Minnesota Agricultural Experiment Station.

[2] Bailey, I. G. Some relations between ants and fungi. Ecology 1: 174–189. 1920.

pellets was not tested. Bailey pointed out the "necessity for further and more detailed investigations upon the structure and feeding habits of the Formicidae and the role of these insects in the dissemination of plant diseases."

Whetzel,[3] in a discussion of Botrytis blight of peony, stated, "Ants seem to carry the spores from the base of diseased stalks to the buds of healthy plants. Here, in the exuded sugary solution, so abundant upon the unopened peony buds, the spores find both food and moisture and germinate much more promptly and vigorously than in water." No mention was made of the method in which the spores are carried by the ants.

The writers have observed ants feeding on the sugary secretion of young peony buds, many of which became necrotic and failed to open. Attempts were made to determine whether the ants were distributing fungus spores in their infrabuccal pockets and, if so, whether the spores were retaining their viability and causing infection. Careful observations by the writers revealed the presence on the buds and stems of many black pellets, later identified as voided infrabuccal pellets. Ants (*Formica fusca* var. *subsericea* Say) were taken from peony buds and the infrabuccal pellets dissected out and examined microscopically. Spores of several different kinds of fungi were found, the most prevalent being *Alternaria*. When the pellets were crushed in hanging drops of sterile distilled water, many of the spores germinated (Fig. 1), showing that viable spores may be disseminated in this way.

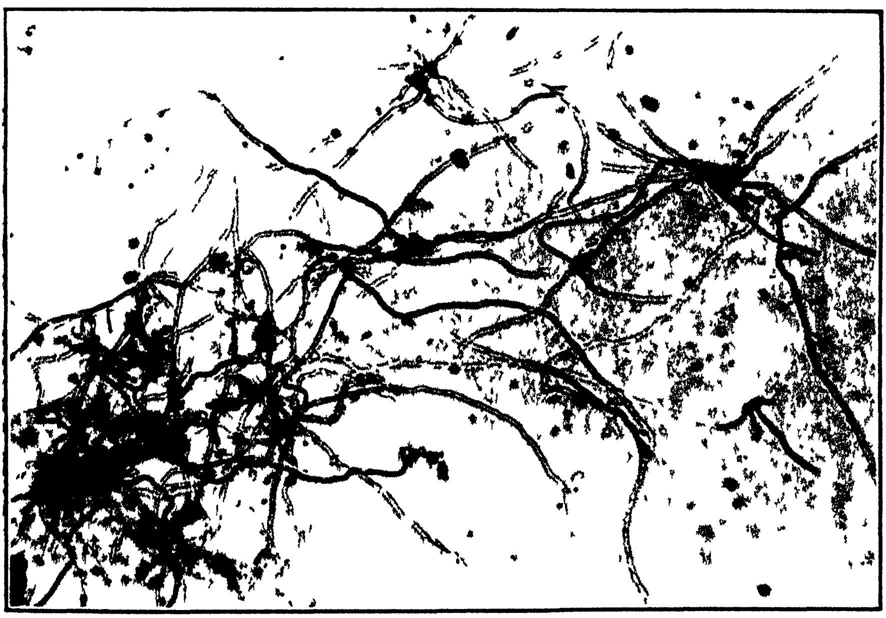
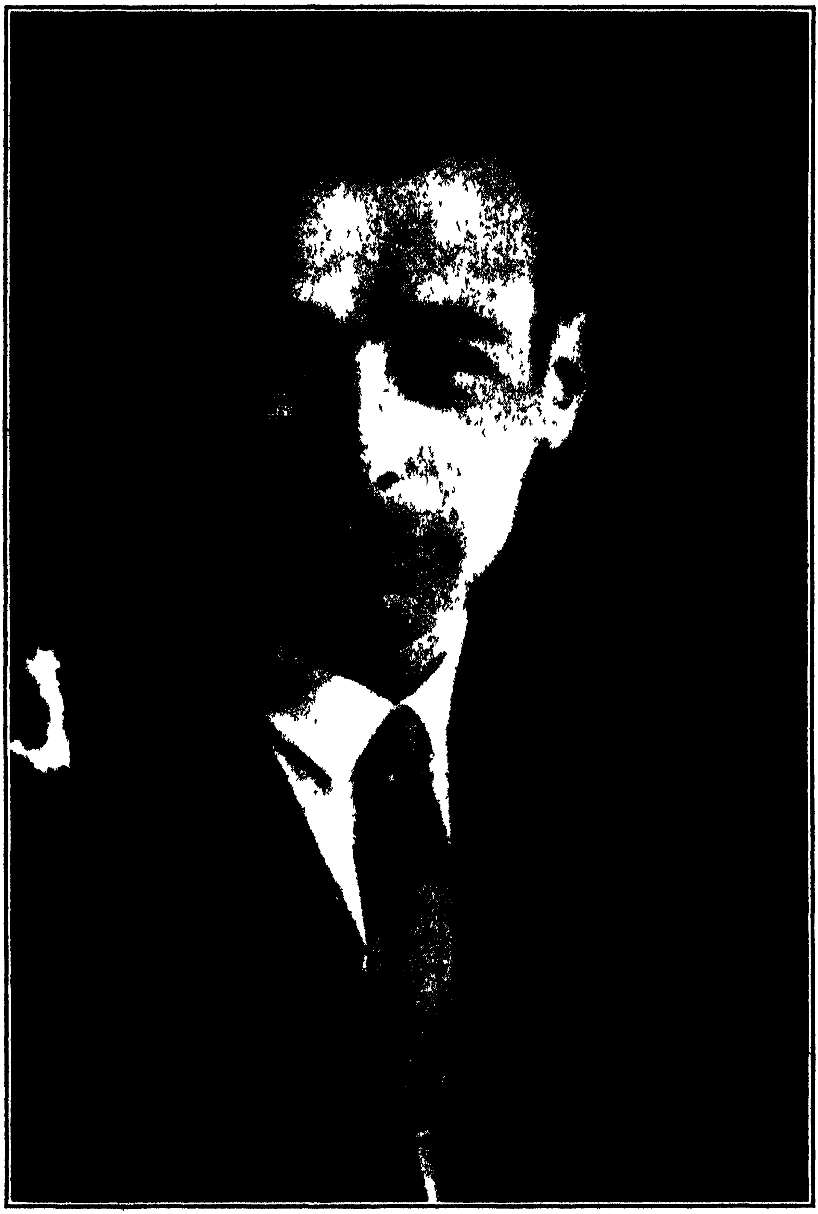


FIG. 1. Mycelium arising from viable spores of *Alternaria* sp. from an infrabuccal pellet of *Formica fusca* crushed in a drop of sterile distilled water.

[3] Whetzel, H. H. Diseases of the peony. American Florist 44: 609–612. 1915.

It was not possible to show any relationship between the fungus spores disseminated by the ants and the blighting of the peony buds. Necrotic petals taken from buds in early stages of necrosis proved to be sterile, indicating that the blight probably was not caused by a parasite. In later stages of development a species of *Alternaria* and an unidentified white hyphomycete were isolated. When young peony buds were sprayed with spore suspensions of these fungi, however, they did not become blighted. Also, when 4 peony plants were protected from ants by means of a metal cylinder and a band of "tanglefoot" glue, the amount of bud blight was not reduced significantly.

These observations show that viable fungus spores are disseminated in the infrabuccal pellet of ants but that the ants associated with peony buds in these experiments were not involved in the development of the bud blight. Peony blights caused by *Botrytis* and *Phytophthora* were not prevalent when these experiments were made, and no spores of these fungi were found in the infrabuccal pellets of the ants or on the blighted buds. Since ants, in general, do not make wounds in plant tissue the likelihood of ants acting as effective vectors of plant diseases is probably less than that of insects that puncture the tissue. Ants that visit flowers or fresh wounds, however, should be looked upon with suspicion.—J. G. LEACH and LOUISE DOSDALL, University Farm, St. Paul, Minnesota. Cooperative investigations between the Divisions of Plant Pathology and Entomology, University of Minnesota.



FRITZ SATTLER
(1905–1937)

FRITZ SATTLER

1905–1937

O. Appel

Am 12. September 1937 starb völlig unerwartet infolge eines Herzschlages in Quedlinburg a. Harz der Phytopathologe Dr. Fritz Sattler.

Er war am 3. Juni 1905 in Ulm a. d. Donau geboren. Von Jugend auf von einer leidenschaftlichen Liebe zu allem, was mit Botanik zusammenhing erfüllt, bahnte er sich unter den allergrössten Schwierigkeiten und persönlichen Opfern den Weg zu seiner Lieblingswissenschaft. Er studierte von 1925 bis 1931 in Tübingen und Freiburg Naturwissenschaften. Im Jahr 1932 begann er in Bonn am Institut für Pflanzenkrankheiten seine Promotionsarbeit über "Thilavia basicola" und führte gleichzeitig Untersuchungen über Viruskrankheiten und deren Übertragung durch Blattläuse aus. Im Jahre 1934 kam er nach Giessen an die Hauptstelle für Pflanzenschutz. Hier beendete er seine Promotionsarbeit und beschäftige sich vorwiegend mit Nematodenkrankheiten der Rüben (*Heterodera Schachtii*), Kohlfliege (*Chortophila brassicae*), Brennfleckenkrankheit an Bohnen (*Gloeosporium Lindemuthianum*), Löffelkrankheit an Azaleen (*Exobasidium japonicum*). Im Frühjahr 1935 legte er nach seiner Doktorpromation als 1. die in Giessen neueingerichtete Prüfung für Pflanzenschutz (Pflanzenarzt) ab.

Danach bearbeitete er im Auftrag des Reichsforschungsdienstes der Landbauwissenschaften an der Abteilung für Pflanzenkrankheiten des Institutes für Pflanzenbau und Pflanzenzüchtung der Ludwigs-Universität, Giessen die Biologie und Bekämpfungsmöglichkeiten des Apfelblütenstechers (*Anthonomus pomorum*). 1937 konnte er bereits seine umfrangreichen Versuche, die sich über ganz Süddeutschland erstreckten mit Erfolg abschliessen.

Im Frühjahr 1937 trat er als Phytopathologe und Saatzuchtleiter in die Saatzuchtfirma Gebr. Dippe A. G. in Quedlinburg ein. Dort beabsichtigte er auch seine Arbeit über Anthonomus pomorum zu vollenden und sie als Habilitationsschrift einzureichen.

Die Gediegenheit seines Wissens, der nie rastende Fleiss, seine ernste Auffassung wissenschaftlicher Arbeit liessen hoffen, dass Sattler bald mit weiteren Arbeiten hervortreten und Tüchtiges leisten würde. Diesen Hoffnungen hat sein Tod ein zu frühes Ziel gesetzt.

Dr. Sattler veröffentlichte:

Zur Biologie von *Thielavia basicola* (Zopf); Phytopathologische Zeitschrift Bd. 10, Heft 1, Jahrgang 1936.

Erneutes Massenauftreten der Runkelrübenmotte (*Phthorimaea ocellatella* Boyd) in Hessen; Nachrichtenblatt für den deutschen Pflanzenschutzdienst.

Der Apfelblütenstecher (*Anthonomus pomorum* L.), vorläufige Mitteilung über die Versuchsjahre 1935 und 1936; n. a. veröffentlicht im "Obstbau" Heft 1, 1937.

Das Ergebnis seiner erfolgreich beendeten Untersuchungen über den Apfelblütenstecher erscheint als vorläufige Mitteilung z. Zt. und der ausführliche Bericht in der Zeitschrift "Der Forschungsdienst" im Laufe des Jahres durch seinen Nachfolger und seine technische Assistentin.

FACTORS INFLUENCING THE PATHOGENICITY OF PYTHIUM DE BARYANUM ON SUGAR BEET SEEDLINGS[1]

W. F. BUCHHOLTZ[2]

(Accepted for publication January 24, 1938)

Seedling diseases constitute one of the chief limiting factors in obtaining maximum stands and yields of sugar beet, *Beta vulgaris* L. Fields with stands of less than 50 per cent occur in Iowa nearly every year. Sometimes entire fields are abandoned for beets and planted to other crops because of high seedling mortality. More frequently the damage is restricted to rather definite field areas.

The work here reported relates to the cause and control of these stand failures. When it appeared that *Pythium de baryanum* Hesse caused at least 95 per cent of the damage, the problem became a study of the biology of this organism in relation to its effect on the host. Such a study has involved, principally, the effect of temperature on the growth and pathogenicity of *P. de baryanum*, its response to pH, its relation to soil acidity and response to liming, and possibilities of control by seed treatment.

PERTINENT LITERATURE

Hellriegel (13) first suggested the parasitic nature of seedling troubles of sugar beets grown in field soil. He came to this conclusion after taking into account various nutritional and other causes, when he found that soaking seed in a weak solution of carbolic acid reduced the amount of disease. He attributed this effect to the disinfection of the seed ball. Frank (9) and Krüger (14) pointed out the importance of *Phoma betae* (Oud.) Fr. as a cause of sugar-beet-seedling disease, but Krüger suggested that the seedling trouble known as "Wurzelbrand" was probably a combination of several diseases involving several pathogens.

So many different causes had been ascribed to this trouble that Peters (19) wrote as follows concerning the matter:

[1] Journal Paper No. J-526 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 482. Taken from a part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[2] These studies were conducted in the plant pathology laboratory of the Iowa Agricultural Experiment Station. The author wishes to express his appreciation to Dr. I. E. Melhus, who proposed the problem, offered many suggestions, and read the manuscript, and to Dr. C. S. Reddy, who also made suggestions during the course of experimentation.

Die Anzahl der angeblichen Wurzelbranderreger ist später noch um einige Pilze und Bakterien vermehrt worden, so dass noch kürzlich als Ursache des Wurzelbrandes nebeneinander Verhältnisse anorganischer Natur, Frass niederer Tiere und Parasitismus verschiedener Pilze und Bakterien angegeben wurden. Unter diesen Umständen schien es geboten die Frage in umfangreicherer Weise, als es bisher geschehen war, auf experimentellum Wege zu untersuchen und die verschiedenen Angaben, die im speziellen Teil eingehender gewürdigt werden sollen, einer experimentellen Nachprüfung zu unterziehen

Peters (18) made his first report in 1906 and in his 1911 article (19) he thoroughly described and differentiated the symptoms brought about by *Pythium de baryanum* and *Phoma betae.* He pointed out very clearly that the former is soil-borne and acts much more quickly than *Phoma betae,* which he found to be seed-borne. Very few cultures of *Rhizoctonia* appeared in his isolations from diseased field-grown sugar beet seedlings, and he experienced difficulty in producing damping off by inoculation with *Rhizoctonia.* According to his observations, *Aphanomyces levis* de Bary, also soil-borne, invaded the young seedlings in a manner similar to that of *Pythium de baryanum,* but acted more slowly and, therefore, produced less severe damage.

Edson (7), working at Madison, Wisconsin, on sugar beets grown in mid-western and western soils, essentially confirmed in 1915 the work of Peters (18, 19). He found *Phoma betae* in his seed stocks but apparently it was not a very aggressive parasite. *Pythium de baryanum* and a new fungus, *P. aphanidermatum* (Edson) Fitzp., were the most destructive parasites involved. Beet seedlings grown on soils from western Kansas and Colorado yielded cultures mainly of *Rhizoctonia.*

Coons and Stewart (5) found that *Phoma betae, Pythium de baryanum* and *Rhizoctonia* were the causal agents of sugar-beet-seedling diseases in Michigan and devoted considerable effort to the development of chemical seed treatments. Previously, Miss Rumbold (21) had reported favorable results from sugar-beet-seed disinfection with formaldehyde and steam. Peters (19), in his work, had soaked seed in water at 60° C. for 10 minutes on two successive days to control seed-borne *Phoma betae.* Coons and Stewart (5) reported that formaldehyde, furfural, nickel carbonate dust, Kalimite, Tillantin B and Seed-O-San were of little or no value in their experiments, but that Semesan, DuPont Semesan 13, Chlorophol, Uspulun and mercury bichloride had given marked stand improvement. Copper carbonate and copper sulphate-lime dusts were intermediate in control value.

Arrhenius (1), working in Sweden, found an association between soil acidity and seedling diseases of beets. He reported that analyses of soil from infested fields showed an acid reaction in every case. The most favorable reaction for the development of beets and the control of their seedling diseases appeared to be pH 7.2 to 7.6, which, he stated, could be secured by applying lime in suitable quantities. Cultural studies with *Pythium de baryanum,* which he considered to be the most frequent cause, showed that the growth of the fungus was almost entirely inhibited at pH 7.5. He also pointed out that more serious damage occurred in later-planted fields, espe-

cially on areas of acid soil. Molz (17) in Germany, Gäumann (11) in Switzerland, Marchal (16) in Belgium, Gallagher (10) in Ireland, Hartwell and Damon (12) at Rhode Island, and others have likewise noted failures of sugar beets on acid soils and improvements in stand and yield from the addition of lime.

THE NATURE AND CAUSAL AGENT OF STAND FAILURES IN NORTHERN IOWA

The severity of losses from stand troubles in northern Iowa is variable. In nearly all fields there is some seedling mortality, but the practice of sowing an excess of seed and selectively thinning usually establishes a stand of 50 to 80 per cent. Even such practices, however, fail to establish stands when damping off is severe. Such a condition is shown in figure 1. In this area less than 20 per cent of a stand was marketable, and the yield was less than a ton per acre. Stands as poor as this seem limited to certain fields, and the most severe damage has been on localized well-defined areas within a field.

FIG. 1. A spot in a sugar-beet field near Kanawha, Iowa, in which the stand was destroyed by damping off in 1932.

Seedlings grown in the greenhouse and in the field in soil taken from such areas exhibit symptoms similar to those shown in figures 2 and 3. In 1932 the examination of many such plants was undertaken. Isolations from a large number of diseased seedlings with 3 or 4 leaves (Fig. 3) resulted in a very low yield of a variety of fungi. It soon became evident, however, that such seedlings probably were not at the stage of primary infection. To prevent excessive contamination of isolation plates by soil bacteria that

FIG. 2. Sugar beet seedlings grown in the greenhouse on soil from the area shown in figure 1. The soil in the pot at the right was steamed.

FIG. 3. Growing sugar beet seedlings of two different ages: the two on the outside healthy, the two toward the center with symptoms of cortical invasion of the hypocotyl by *Pythium*.

had invaded these advanced lesions, it was necessary to surface disinfect so heavily that there was also a possibility of killing the pathogen if it were still present. In subsequent isolation trials, water-soaked but not collapsed portions of recently invaded younger seedlings were placed on potato-dextrose agar plates after very little, preferably no surface disinfection with mercuric chloride. Such a procedure seldom yielded any fungus cultures other than of *Pythium*. The same method yielded cultures of *Phoma betae* from infected seedlings grown from nontreated seed on steamed soil in the greenhouse and, on rare occasions, from field specimens that were not entirely killed by *Pythium* or that were grown under conditions (such as low temperature) unfavorable for *Pythium*.

Cultures of *Rhizoctonia* have rarely been recovered from field-grown seedling beets. Such cultures, introduced into steamed soil in the green-

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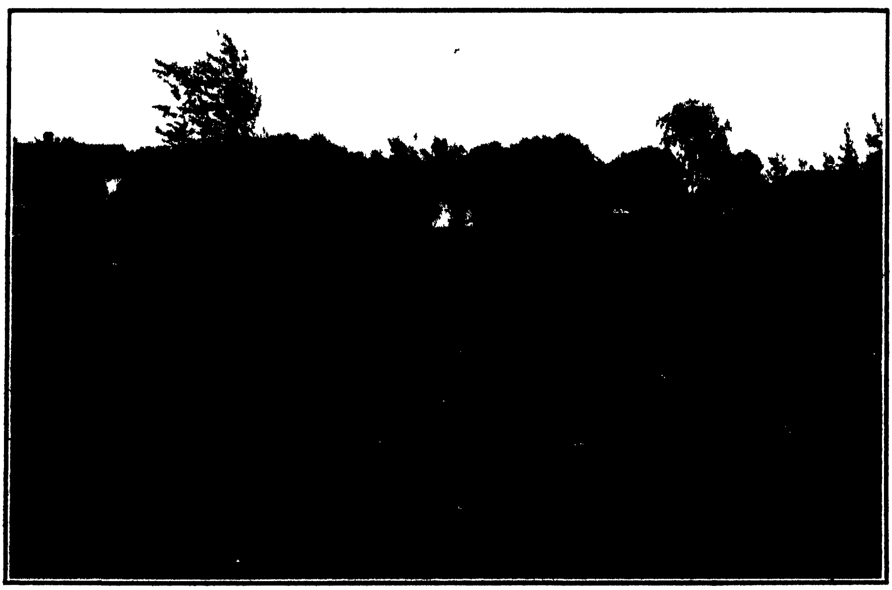

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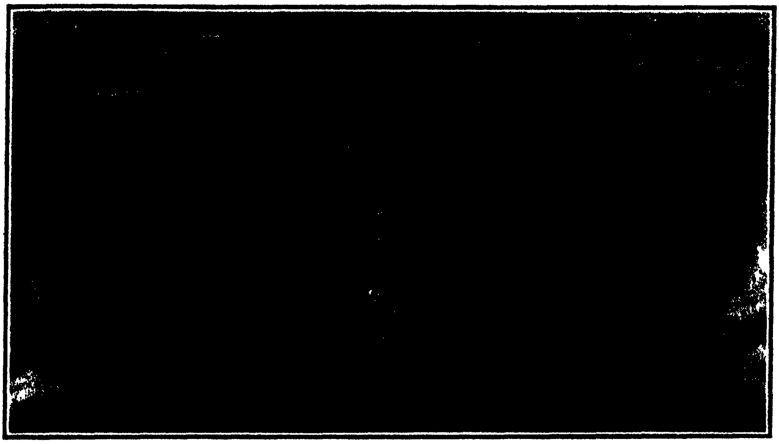

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Cultures of *Rhizoctonia* have rarely been recovered from field-grown seedling beets. Such cultures, introduced into steamed soil in the green-

house, have induced necrotic lesions on seedlings. It seems improbable that numerous isolation tests would have yielded so few *Rhizoctonia* cultures if this pathogen were responsible for considerable damage in the field.

Prevalence and Development of Phoma betae, Pythium and Rhizoctonia

Numerous isolations from diseased seedlings grown in the field and in the greenhouse resulted in the recovery of *Pythium* from a large majority of both field and greenhouse specimens. Subsequent inoculations showed that such cultures of *Pythium* were highly pathogenic. Cultures of *Rhizoctonia,* while also pathogenic, were not so virulent, and were seldom recovered from either field- or greenhouse-grown seedlings. *Phoma betae* proved to be even less pathogenic than *Rhizoctonia* and was recovered infrequently, except from seedlings grown from infected seed on steamed soil in the greenhouse.

To observe and compare the relative pathogenicity of *Pythium, Rhizoctonia* and *Phoma betae* under controlled conditions, cultures of *Pythium* and *Rhizoctonia* were introduced into steamed soil in the vicinity of germinating or emerging sugar-beet seedlings grown from *Phoma betae*-infected seed at the same time that sugar beets were growing from the same lot of seed on several field soils and noninfested steamed soil, as follows:

Two rows of 10 sugar-beet seed balls were planted in each of 32 pots of steamed Clarion loam with a 2-ton lime requirement. In 8 of the pots, 1 row of seed was treated before planting with ethyl mercuric phosphate dust (5 per cent, ¼ g. per 100 g. of seed). In 8 others, 2 small portions of a pathogenic *Rhizoctonia* culture were placed in contact with the seeds in 1 row at the time of planting. In 8 others the *Rhizoctonia* culture was placed in contact with 1 row of emerging seedlings, as were 2 similar portions of a pathogenic *Pythium de baryanum* culture, in the remaining 8 rows. In each pot, 1 row was left without either treatment or inoculation to serve as a check.

In like manner, in each of 8 pots of nonsteamed Clarion loam from Kanawha, 8 pots of nonsteamed Clarion loam from Ames, and 16 pots of nonsteamed, neutral Webster silty clay loam from Ames, were planted 1 row of 5 treated and 1 row of 5 nontreated seed balls. Fewer seed balls were planted in the nonsteamed field soil to avoid excessive spread of the pathogen from one group of seedlings to another, so that the disease counts would more nearly estimate the actual soil infestation. The counts were then doubled to make them comparable in value to those taken from the steamed soil.

Two readings were taken, the first on March 11 as soon as seedlings in the steamed Pythium-infested soil began to die; the second on March 18 and 19, when the seedlings were dug (Table 1). At the first reading, the nontreated rows on the nonsteamed Clarion loam had the poorest stand of any in the experiment. Rhizoctonia infestation at planting time also resulted

in a poor stand, but infestation at emergence had not produced any disease, whereas about 20 per cent infection had resulted from Pythium infestation. Almost no symptoms of *Phoma betae* infection were visible on seedlings grown from nontreated seed in steamed soil. At the second reading, there were only occasional healthy seedlings from nontreated seed on nonsteamed Clarion loam and in the Pythium-infested rows on steamed soil. By this time Rhizoctonia infestation at emergence had produced considerable infection, and the earlier infestation had spread across to the check row. Considerable *Phoma betae* infection also had developed on seedlings from nontreated seed in steamed soil. This was largely controlled by the ethyl mercuric phosphate dust, as was the Pythium infection on nonsteamed Clarion loam. On nonsteamed Webster silty clay loam, there was no more disease than on steamed soil.

Results of this experiment confirmed the previous field and greenhouse observations that were supported by isolations and subsequent infestation and inoculation trials similar to parts of this experiment.

Symptoms of Phoma betae infection. In this experiment, *Phoma betae* caused evident injury only in the steamed soil and in the Webster silty clay loam, which apparently was only lightly Pythium-infested. In both cases it was controlled by seed treatment. *Phoma betae* has been commonly found in seed lots and observed to infect sugar-beet seedlings grown in steamed soil. It eventually will produce an extensive cortical lesion, but, for at least 10 days after emergence of the seedling under greenhouse conditions, there was visible only a constantly darkening tan to brown area at the base of the hypocotyl, which developed progressively from the point of contact with the seed ball. Sometimes a brown necrotic streak extended up and down from what appeared to be the point of infection. The mycelium is at least temporarily restricted to the intercellular spaces and the infected hypocotyl is more brittle than is normally the case.

Gradually, the invaded cortex turns black and, in many instances, collapses. The plants may fall over, but, frequently, under favorable soil and other conditions, continue to live and grow. Apparently, the invading hyphae seldom penetrate to the vascular cylinder and, if growth conditions are sufficiently favorable, an infected hypocotyl may continue to thicken despite the condition. Eventually, the dead cortical tissue disintegrates and is replaced by secondary tissue. In the greenhouse this process ordinarily took 2 or even 3 weeks after the emergence of the seedling.

Symptoms of Pythium infection. If *Pythium* is abundant and active in the soil, it may destroy 95 per cent of the seedlings before seed-borne *Phoma betae* makes any progress (Table 1). The pure-culture infestation with *Pythium de baryanum* made on March 9 brought about the infection of more seedlings in 2 days than were found to be infected with seed-borne *Phoma betae* 10 days after emergence. Many of the seedlings in Clarion loam did not emerge. Some were invaded in the seed ball. This has not been the case in the field, where many have been invaded shortly after appearing outside

TABLE 1.—*Average number of healthy and diseased sugar-beet seedlings from greenhouse-planted rows of Phoma betae-infected seed in steamed soil, in soil naturally infested with Pythium and soil artificially infested with Pythium de baryanum and Rhizoctonia*

Soil	Average number of healthy and diseased seedlings											
	March 11						March 18 and 19[a]					
	Row of treated seed[b]		Check		Row in artificially infested soil		Row of treated seed		Check		Row in artificially infested soil	
	H	D	H	D	H	D	H	D	H	D	H	D
Neutral Webster silty clay loam, not steamed	27	0	26	0.5			28	0	23	3		
Acid Clarion loam, not steamed	26	0	6	3			24	4	2	7		
Acid Clarion loam, steamed	27	0	25	0			26	1	21	5		
Acid Clarion loam, steamed:												
Rhizoctonia infestation, March 2			25	0	12	1			13[c]	13	8	6
Rhizoctonia infestation, March 9			25	0	26	0			22	5	11	16
Pythium infestation, March 9			24	0	19	5			21	4	4	20

[a] Seedlings dug up at this time.
[b] Seed treated with ethyl mercuric phosphate dust, 5 per cent, $\frac{1}{4}$ g. per 100 g. of seed.
[c] Infestation had spread across pot to noninfested row.

the seed coat or shortly before emerging from the soil. In these cases, and in many instances of infection of emerged seedlings, the invasion extends into the vascular, as well as the cortical, tissue so that wilting ensues and the seedlings die quickly. Young, emerging seedlings, when thus infected, rapidly become desiccated, especially in the field.

The initial Pythium infection appears as a slightly darkened, glistening, water-soaked spot, usually occurring about ½–2 inches below the surface of the soil, depending on moisture level and depth of planting. The region of the hypocotyl, just at and above the base of the collet, seems to be the most susceptible part of the young seedling. A result of invasion of the vascular cylinder is a blackening of that portion of the axis, even beyond the extent of the cortical lesion. Young seedlings so invaded die quickly. When no vascular invasion occurs, the seedlings may continue to live and grow (Fig. 3). As it persists, the growing cortical lesion first turns to a chestnut brown, then collapses, and turns black. Sometimes it extends to the base of the cotyledons. The rapidity of such extension as compared to the general vigor and rate of growth of the seedling apparently determines the ultimate fate of the plant.

Occasionally, the central axis may be severed and the plant yet remain alive. Under conditions of sufficient moisture, roots may develop from the lowermost portion of the living axis just above the severed region. Such individuals apparently become what might well be called "turnip" beets. They are undersize, short, round, and usually develop several to many branch roots. Commercially, they are worthless, rarely attaining a weight of ¼ pound.

Symptoms of Rhizoctonia Infection. Rhizoctonia injury of seedlings, under northern Iowa conditions, is difficult to evaluate or describe. It was rarely obtained in isolations from field-grown diseased seedlings, especially very young ones. Some cultures have proved pathogenic to small seedlings, while others have brought about but little injury. Rhizoctonia lesions in the above experiment differed from those induced by *Pythium* in that they became a dark brown or chocolate, shortly after infection, and were less water-soaked.

It is not particularly difficult to recognize the above described distinctions between the types of symptoms produced by these 3 different pathogens when the mass effects of each are available for observation, but in the field it is not possible to determine the particular organism involved in the individual cases without resort to isolations.

Under northern Iowa conditions, at least 95 per cent of the damage to seedling sugar beets appears to be due to *Pythium*. This statement is supported by the following findings: (1) Isolations from young seedlings grown in the field or on field soil in the greenhouse give almost 100 per cent *Pythium* cultures unless seedlings were grown in cold or lightly Pythium-infested soil; (2) *Pythium*, which occurs naturally in field soil, and that introduced into steamed soil is much more rapid and destructive in its action

than either *Rhizoctonia* or seed-borne *Phoma betae;* (3) symptoms that occur on seedlings known to be infected with *Pythium* in the greenhouse are similar to the symptoms observed on young seedlings in the field.

Identity of the Pythium Isolates from Sugar Beets

Although not identical, a majority of the *Pythium* isolates, taken from diseased sugar beet seedlings grown in northern Iowa were enough alike to be considered the same species. Their young mycelium branched freely, was nonseptate, relatively hyaline, and from 1.5 to 3.5 μ in diameter. Older mycelium was larger and septate, and seemed to be devoid of protoplasm. When a portion of a culture was transferred to sterile distilled water, spherical or slightly ovate conidia, 10 to 35 μ in diameter, were found terminally on branches of the newly-formed mycelium. Similarly formed, intercalary conidia were of the same size and spherical to ellipsoid in shape. If chilled at 0°–10° C. for 1–6 hours and then transferred to carrot decoction or malt extract, the conidia germinated by a germ tube in 15 to 20 minutes to 4 hours at room temperature, the time being fairly characteristic of a given culture. Zoospore production was not observed.

Occasionally oospores were produced simultaneously with, or soon after, the formation of conidia from cultures in distilled water. They formed more readily in the tissues of an infected host plant that had been left floating in distilled water 4–7 days. The oospores averaged 13.6–17 μ in diameter; and some contained a central globule. The oospore walls were smooth and varied in thickness from 1.5–2 μ. The mature oospores were free in the oogonium. The oogonia averaged from 16.6–22.1 μ in diameter; their thin walls were membranous and smooth. Not more than 2 androgynous antheridia were observed with a single oogonium.

With the exception of the failure of zoospore formation, which seems also to have been the experience of Atkinson (2), Ward (22), and Butler (3, pp. 86–91), the characteristics of the *Pythium* isolates taken from diseased sugar-beet seedlings grown in northern Iowa would classify them in the species, *Pythium de baryanum* Hesse; and it seems probable that stand failures, in northern Iowa, are caused by the same organism, encountered first by Peters (19), in Germany, and later by Edson (7) and Coons and Stewart (5) in the United States.

TEMPERATURE RELATIONS OF PYTHIUM DE BARYANUM

It is a common experience among beet growers in northern Iowa that early plantings produce better stands and higher yields, so much so that the observing and successful growers rarely delay their plantings later than May 10; the most consistently successful growers rarely fail to plant in April. These observations suggested the possible relation of soil temperature to the abundance and pathogenicity of *Pythium de baryanum* in the soil.

Temperature and Growth Rate in Pure Culture

A pathogenic culture of *Pythium de baryanum* was subjected to each of 9 different temperatures in the following manner: In the center of each of 54 potato-dextrose agar plates was planted a small piece of agar with rapidly growing mycelium. These plantings were allowed to stand at room temperature for 8 hours to become established and commence to grow. At this time the colonies were 1 to 2 cm. in diameter. Their margins were plotted on the glass at 4 points. Subsequently, the cultures, 6 at each temperature, were incubated at 5, 9, 12, 15, 20, 25, 30, 35, and 40 degrees C. for 12 hours. Two measurements of increase in diameter of each colony were averaged to yield the data shown in figure 4. There appears to be a rather distinct upward break in the curve between 12° and 15° C. Maximum growth was obtained at 30° C., with a very sharp drop at 35° C.

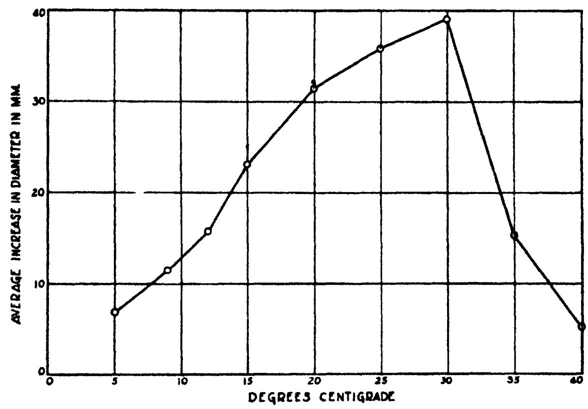

FIG. 4. Growth of *Pythium de baryanum* in 12 hours at various temperatures.

It seemed that neither the maximum nor the minimum temperature had been included in the series. Furthermore, growth was so rapid that some increase in diameter probably took place in plates at the low and high temperatures before the entire mass of 6 plates had been adjusted to those temperatures. Accordingly, the entire experiment was repeated in the same manner, except that after growth had started the plates were allowed to remain in the respective incubators approximately 2 hours before the margins were first plotted. With this procedure no growth occurred on any of the plates at 1° and 5° C., and a measurable growth on only one of the plates at 40° C. Otherwise, the results were similar to those described in the previous experiment.

Temperature and Pathogenicity in Field Soil and in Pure Cultures

A series of experiments was designed to determine the relative amount of emergence and damping off of beet seedlings grown in naturally infested field soil and artificially infested steamed soil at various temperatures. In the first of these, 36 pots of naturally infested field soil, planted with 5 seed

balls each, were incubated, 4 at each of 9 temperatures, 5, 9, 12, 15, 20, 25, 30, 35, and 40 degrees C. All were adequately watered, but no attempt was made to maintain a constant moisture content. No light was available to the seedlings after emergence. Emerged and diseased seedlings were counted daily until the plants at the higher temperatures were all dead or very etiolated and abnormal; then they were discarded and subsequent counts were taken every other day on those at the lower temperatures. As soon as an individual seedling showed infection it was removed to avoid spread of the pathogen from it to others near-by. *Pythium* was recovered from diseased seedlings at all temperatures (Table 2). The numbers, emerged and diseased, are shown in figure 5.

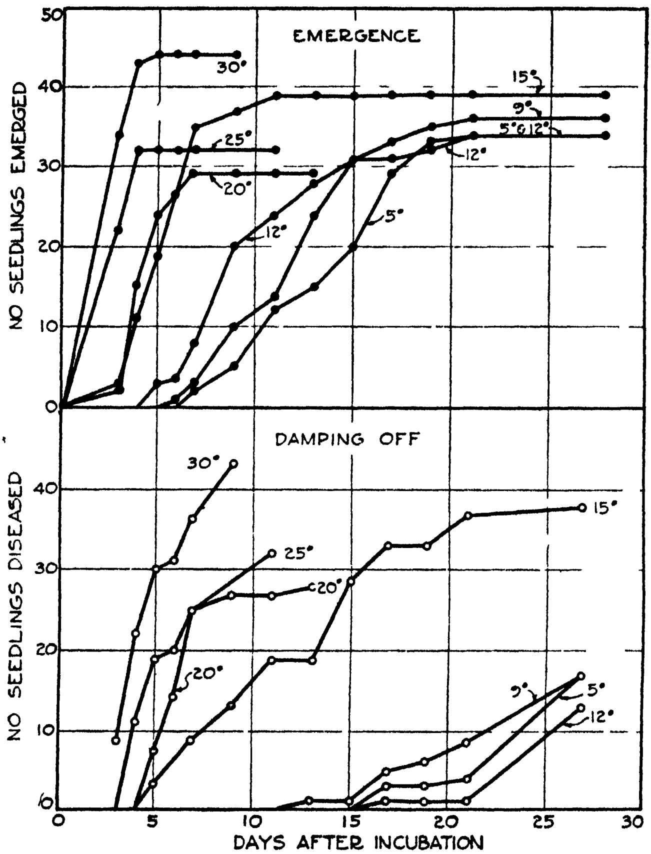

FIG. 5. Rate of emergence and damping off of sugar-beet seedlings grown on naturally infested field soil at various temperatures (C).

It is evident that at 5°, 9°, and 12° C., while the rate of emergence and damping off were both slow, the latter was enough slower that, when the experiment was discontinued, some plants were still healthy. At temperatures of 20°, 25°, and 30° C., infection closely followed rapid germination, and at the time of discontinuation only one healthy plant remained at 30° C. The data taken at 35° and 40° C. were not included in figure 5 because, at

these temperatures, the seedlings quickly became extremely etiolated. Nevertheless, the pots at these temperatures did yield less damping off than did those at 20°, 25°, and 30° C.

No explanation can be offered for the apparently slow rate of damping off at 12° C. The 15° C. incubator fluctuated upward several times, so the germination rate and damping off represented for that temperature may be a little high. Even so, the incidence of disease at 12° C. is below 9° and 5° C.

TABLE 2.—*Recoveries of Pythium from diseased seedlings grown in field soil at different temperatures*

Temperature °C.	No. seedlings plated	No. times Pythium recovered
5	17	4
9	17	15
12	13	10
15	20	14
20	13	11
25	12	12
30	12	12
35	12	12
40	8	5
Total	132	95

In a second experiment, a duplicate of the first, except that 35° and 40° C. incubations were omitted and a different collection of infested field soil was used, emergence and damping off at 12° C. were intermediate between 9° and 15° C. In the latter experiment, however, fewer seedlings emerged at lower temperatures, which was not the case in the experiment shown in figure 5. The lag in damping off, compared to emergence, was again evident at the lower temperatures.

In a third experiment, 8 pots of a third collection of naturally infested field soil and 8 pots of steamed, artificially infested soil, each planted with 5 graded seed balls, were incubated at 5, 10, 15, 20, and 30 degrees C. The emerged and diseased seedlings at each temperature were counted when it was thought no more emergence would take place. All seeds were dug up and unemerged seedlings also were classified as healthy or diseased. The comparisons appear in figure 6.

It will be noted that the field soil used in this experiment produced less damping off than occurred in the previous experiments. Nevertheless, the total amounts and percentages of damping off on field soil were greater at the higher temperatures. The results on steamed soil artificially infested with *Pythium de baryanum* are especially noteworthy. Infestation was accomplished by covering the seed, which had previously been planted on the surface of the steamed soil in a pot, with a ¼–½ ich layer of steamed soil that had been inoculated in a flask 2 weeks earlier with an agar culture of *Pythium de baryanum*. The infestation apparently was so heavy that at 15°, 20°, and 30° C. very few of the seedlings emerged, and all of those that

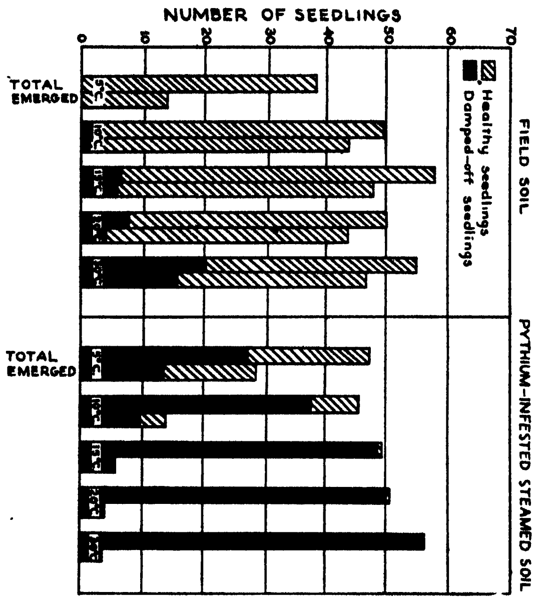

FIG. 6. Numbers healthy and diseased of total (nonemerged plus emerged) and emerged sugar-beet seedlings grown in field soil and Pythium-infested steamed soil at various temperatures.

did became infected. At 5° C. there was about 50 per cent emergence, and of the resultant seedlings nearly one-half were healthy. The results at 10° C. were intermediate. In both field soil and infested steamed soil there were some healthy seedlings that had not emerged, especially at 5° and 10° C.

In all three of the experiments just described, temperatures of 15° C. or below were more unfavorable to damping off than to germination and emergence. This evidence supports the experience of growers with early plantings and has been further substantiated in results from field experiments described elsewhere in this paper.

SOIL FACTORS ASSOCIATED WITH DAMPING OFF IN THE FIELD

The severe damage in rather well-defined areas (Fig. 1) is one of the striking features of damping off of sugar beets in the field. On part of a field the stand may be satisfactory, while only a few rods away there may be a complete stand failure. Data taken in 1932 from 2 such adjacent areas are presented in table 3.

Around Kanawha such heavily infested areas usually consist of Clarion loam or Webster loam. They are slightly more elevated than the lightly infested areas, which fall on the lower edge of the Webster silty clay loam and on the Lamoure silty clay loam. The heavily infested soil appears to be somewhat lower in organic matter and not so loose and friable, but to all appearances it produces nearly as good crops of corn and small grain as does the lightly infested soil.

TABLE 3.—*Sugar-beet stands harvested in 1932 from adjacent areas in the same field at Kanawha, Iowa.*

Heavily infested soil area			Lightly infested soil area		
Stand	Market-able beets	Perfect stand mar-etable	Stand	Market-able beets	Perfect stand mar-ketable
Per cent[a]	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
50	32	16	102	98	100
44	45	20	64	100	64
54	7	4	58	96	56
56	43	24	66	100	66
36	67	24	106	94	100
Ave. 48	38.8	17.6	79.2	97.6	77.2

[a] One beet per linear foot of row was considered a 100 per cent drilled stand. Beets with a root diameter of 1 inch or more were classed as marketable.

Soil Moisture in Areas of Good and Poor Stands

It was thought that the high percentage of damping off in these heavily infested areas might be the indirect result of excessive soil moisture, even though these areas are on gentle slopes or at the tops of knolls. In the fall of 1932, soil moisture determinations were made at various depths to 30 inches in 4 areas, 2 with good and 2 with poor stands. Duplicate borings were made with a soil auger and the moisture content was determined by the standard procedure, on an oven-dry basis. The moisture content of the surface six inches was slightly higher where stands were good than where thin, although on the basis of percentage of saturation the difference was only 1.35 per cent. There was very little difference in subsoil moisture.

In 1934, surface-soil moisture determinations were made twice a week on 2 locations in an experimental plot, one where the stand was good, the other where it was poor. The average moisture content of the latter (I) was 21.89 per cent, for the former (II), 23.13 per cent (Table 4). The moisture-holding capacity was 49.55 per cent in Area I and 55.55 per cent in Area II, so that on the basis of percentage saturation, Area I was more moist on the average than Area II. For a short period following rains, the soil appears noticeably more moist in Area I than in Area II. Sugar-beet seeds germinated more quickly in Area I. A comparison of the data obtained by averaging all the determinations in which both areas had a moisture content of 20 per cent of dry weight or more and all determinations in which one or both of the areas was below 20 per cent shows that at the higher moisture contents, the difference between the averages for the two areas is only 0.94 per cent (Table 4). At the lower contents, the difference is 1.63 per cent. On the basis of percentage saturation, Area I is 4.74 per cent more moist at the higher moisture contents, but actually 0.26 per cent less moist at the lower moisture contents. It may be that during periods of high moisture following considerable precipitation there is more free water in the soil of

Area I, even though the actual moisture content may be no greater or even slightly less. Even so, it is not probable that a difference even as large as 4.74 per cent during periods of high moisture would mean the difference between a good stand and a complete failure, as has repeatedly been the case in these adjacent areas.

Relation of Damping Off to Soil Acidity

In the fall of 1932 in 3 different fields at Kanawha that had poor stands of sugar beets, the soil in these areas had a lime requirement of ½ ton to 3 tons per acre. On adjacent neutral areas in the same field, stands were good. In one case, when sugar beets were planted on such soils in the greenhouse, only 28 healthy plants remained in 16 rows on acid soil, as against 372 on the neutral soil.

TABLE 4.—*Average soil moisture contents of an area with poor stand (I) and an area with good stand (II)*

Moisture content	Area with poor stand (I)		Area with good stand (II)		Difference (I)-(II)	
	Dry wt.	Saturation	Dry wt.	Saturation	Dry wt.	Saturation
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Average of 24 high-moisture contents	29.52	59.57	30.46	54.83	- 0.94	4.74
Average of 19 low-moisture contents	12.25	24.72	13.88	24.98	- 1.63	- 0.26
Average of 43 moisture contents	21.89	44.17	23.13	41.63	- 1.24	2.54

During the 1933 season a field survey was made in Hancock, Wright, and Cerro Gordo counties, including especially those fields that showed good and poor stands of beets in adjacent areas. One composite soil sample together with 4 counts of thinned stand were taken in each area of poor and good stand. Lime requirement and pH determinations were made in duplicate for each soil sample. Lime requirement determinations were made with the Emerson (8) soil-test solution. When this solution is mixed with an acid field soil, a red color is produced, and the lime requirement of the soil is estimated by comparison with a standard color chart. Determinations of pH were made with Lamotte indicators and standards, after the method of Clark and Lubs (4). A measured amount of supernatant solution from a settled mixture of soil and distilled water was decanted off, a standard amount of indicator added, and color comparisons made. Occasionally a sample was slightly cloudy, but by the use of similar checks in the comparator block it was possible to estimate to the nearest one-tenth of a pH value. The indicators most commonly used were brom thymol blue (pH 6.0–7.6), chlorphenol red (pH 5.2–6.8), and, occasionally, phenol red (pH 6.8–8.4).

TABLE 5.—*Percentage stands of sugar beets on northern Iowa soils with and without lime requirements in 1933*

No. soils	Lime requirement, tons per acre		Soil pH range	Percentage stand	
	Average	Range		Average	Range
21	1.89		(Not determined	47.04	
17	0.00		for 10 samples)	76.26	
15 (See	2.06	0.5–3.0	5.95–6.5	41.4	10.5– 75.0
13 text)	0.00		6.4 –7.6	79.15	38.5–107.5
14	2.04	0.5–3.0	5.95–6.5	39.0	10.5– 64.2
12	0.00		6.4 –7.6	82.54	60.0–107.5

Correlations calculated from lime requirement, soil pH and percentage stand data

Correlated variables	Correlation coefficient	Significant values
Lime requirement and percentage stand	−0.556	0.325 0.418[a]
Soil pH and percentage stand	0.745	0.374 0.478[a]
Lime requirement and soil pH	−0.721	0.349 0.449[a]

[a] Highly significant.

Soil pH values, average lime requirements and stand counts, and correlation coefficients are presented in table 5. They show that in the fields visited there was a highly significant correlation between soil acidity and

TABLE 6.—*Lime requirement, soil pH determinations, and thinned stand counts taken in 1934 survey of sugar beet fields in northern Iowa*

Lime requirement, tons per acre	Soil pH	Percentage stand
	Soils with pH 6.5 or less	
2	6.0	55
1	6.45	68
2	6.0	68
0.5	6.3	73
1	6.35	33
2	6.05	24
1.5	6.1	62
1	6.5	50
1.5	6.25	36
2	6.2	38
Average 1.45		50.7
	Soils with pH 6.6 or more	
0	6.65	79
0	7.25	100
0	7.1	92
0.5	6.6	73
0	6.7	67
0	6.85	63
0	7.0	69
0	6.95	95
0	6.9	85
Average 0.055		80.3

poor stands. The lime requirement test divided these soils approximately into 2 groups, those showing a lime requirement and a pH of 6.5 or less, and those without a lime requirement and a pH of 6.4 or more. With one exception in each group, the soils without a lime requirement had a stand of 60 per cent or more and those with a lime requirement had a stand of 64.2 per cent or less.

In 1934 the same procedure (Table 6) was carried out. Not so many fields were visited as in 1933. From the table it is again evident that the more acid soils had poorer stands.

Effect of pH on the Growth Rate of Pythium de baryanum

When the possibility of a correlation between soil acidity and poor stands caused by *Pythium de baryanum* presented itself, it was first thought that the pathogen probably was distinctly favored by an acid medium. Accordingly, several lots of potato-dextrose agar were adjusted with lactic acid and sodium hydroxide to different degrees of acidity and alkalinity ranging from pH 4.0 to pH 8.1. Four plates were poured for each pH value and transfers were made in duplicate for each pH value of two pathogenic cultures of *Pythium de baryanum*. Twelve-hour growth increments were recorded by measuring increase in radius of each plate culture in four places. Results appear in figure 7.

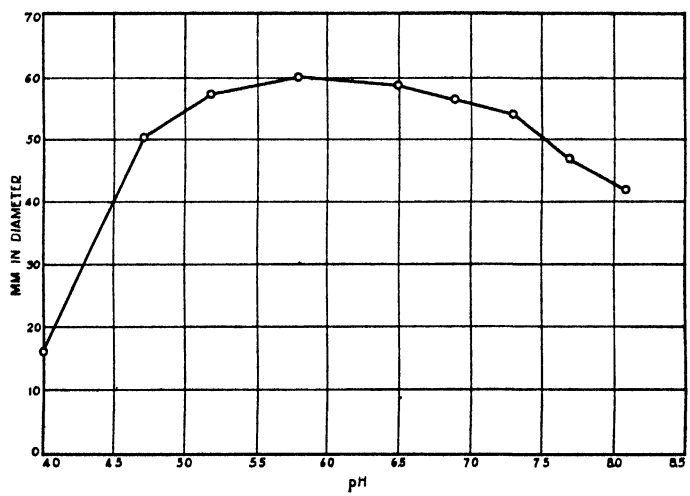

FIG. 7. Growth of *Pythium de baryanum* in 12 hours on potato-dextrose agar adjusted to pH 4.0–8.1.

The most growth occurred at pH 5.8, which would indicate a tolerance for a slight degree of acidity. However, growth at pH 4.7, 5.2, 5.8, 6.5, 6.9 and 7.3 was very nearly the same (between 50 and 60 mm.), and growth at pH 8.1 was greater than at pH 4.0. If these results are indicative of the growth of *Pythium de baryanum* in the soil, one would expect soils with pH values from 4.7 to 7.3 to contain approximately equal amounts of *Pythium*.

The evidence already presented points to the fact that there was more damping off of sugar beets in acid soils.

FIELD RESULTS WITH LIMING, SEED TREATMENT, AND DATE OF PLANTING

The preliminary field observations and greenhouse and laboratory data, thus far presented, have emphasized the relation of soil acidity and soil temperature to the abundance and pathogenicity of *Pythium de baryanum*, the chief cause of stand failure in the field, and have suggested the possibility of control by seed treatment. Field experiments, therefore, were designed to test the value of liming acid and neutral soils, early planting to take advantage of low soil temperature early in the spring, and seed treatment. On Dec. 23, 1932, sugar factory lime cake was applied at the rate of 1.25 tons per acre, air-dry weight, one each of 4 strips of soil that alternated with 4 nonlimed strips. The entire plot extended across an acid and a neutral soil area. On April 24, 1933, 1 ton per acre was added to the previously limed strips and the first planting was made on April 29 on 6 of the strips, 3 limed and 3 not.

This plot had been divided into 14 smaller plots, each of which crossed all 6 strips. Six plots were on acid soil, 6 on neutral soil, and 2 in an intermediate position. The plan was to plant on each plot 1 replication of a

TABLE 7.—*Seed treatments applied in the field experiment of 1933*[a]

Number	Toxic agent	Adhesive properties
1	Ethyl mercuric chloride, 2 per cent	Very good
2	Ethyl, mercuric phosphate, 5 per cent, ½ g. per 100 g. of seed	Excellent
3	Ethyl mercuric phosphate, 5 per cent, 1 part, plus 3 parts hydrated lime	Good
4	Ethyl mercuric chloride, 2 per cent, 50 g. Mercuric chloride 2 g. Hydrated lime 48 g.	Intermediate
5	Ethyl mercuric phosphate, 5 per cent, 20 g. Mercuric chloride 2 g. Hydrated lime 78 g.	Intermediate
6	Ethyl mercuric chloride 10 g. Hydrated lime 90 g.	Intermediate
7	Hydrated lime dust (dry seed balls rolled in excess of dust)	Fair
8	Hydrated lime wet roll (wet seed balls rolled in excess of hydrated lime and allowed to dry)	
9	Diphenyl mercury, 2 per cent	Fair
10	Ethyl mercuric chloride, 2 per cent, 33⅓ g. Mercury furfuramide, 5 per cent, 66⅔ g.	Intermediate
11	Formaldehyde dust	Poor
12	Ethyl mercuric phosphate 25 g. Colloidal phosphate 75 g.	Good

[a] All dust treatments applied at rate of 1 g. per 100 g. seed balls, except treatments 2 and 7. Results in table 9.

sugar-beet seed-treatment experiment, each replication to consist of 4 check rows and 1 row of each of 12 seed treatments. Graded seed balls were used, 1 per hill, in hills 1 foot apart. The rows were 2 feet apart. Twenty-one hills were marked off in the center of each 28-foot strip for counting. Emergence and survival counts were to be taken at intervals, and stand counts and yields at harvest time. The seed treatments used are enumerated in table 7.

Five replicate plots were planted on April 29 and May 1, 2 on acid soil, 2 on neutral soil, and 1 in an intermediate position. A second similar planting was made on May 16. Emergence data had just been collected on the first planting when, on May 23, both plantings were destroyed by wind. From the last planting, made on May 26, emergence and a set of survival data were taken before it became evident that there would not be a measurable yield. The experiment was then plowed up. Soil temperature and rainfall data for the time of germination and emergence for the early (April 29, May 1) and the late (May 26) plantings appear in table 8.

TABLE 8.—*Soil temperatures and rainfall during germination and emergence period for early (April 29, May 1) and late (May 26) plantings in 1933*[a]

	Date planted	Date of emergence reading	Rainfall, inches	Soil temperature, °C.				
				a. m.		p. m.		Ave.
				Ave.	Range	Ave.	Range	
Early planting	Apr. 29–May 1	May 18	2.25, during 9 days	8.5	5.6–13.6	11.3	7.5–14.7	9.89
Late planting	May 26	June 7	0.8 + during 3 days	19.0	12.5–24.0	26.2	16.5–33.0	22.2

[a] Refer to table 9.

Field Results, 1933. While it was evident that no satisfactory control had been devised, some of the data collected indicated possibilities. Comparative total emergence from the early and late plantings is shown in table 9.

It is observed that more seedlings emerged from the early planting than from the late planting, especially on acid soil; the late planted check yielded 50 per cent more emerged seedlings on neutral soil than on acid soil; the early planted check yielded nearly as many emerged seedlings on acid soil as on neutral soil; in the late planting the best seed treatment (No. 1) increased emergence 37 per cent on acid soil and only 7 per cent on neutral soil; in the early planting the best seed treatment increased emergence 52 per cent on acid soil and 34 per cent on neutral soil; and liming did not affect emergence in the early plantings, but did bring about an increase of 12.5 per cent on acid soil and a 4.6 per cent increase on neutral soil in the late planting.

Table 10 contains emergence data from a slightly different combination of the late planted plots, with one set of counts seven days after emergence.

TABLE 9.—*Number of sugar-beet seedlings emerged on limed and nonlimed acid and neutral soil from early and late plantings of treated and nontreated seed, 1933*

Seed treatment[a]	Planting date[b]	Acid soil		Neutral soil		Totals	Rank of seed treatments
		Non-limed	Limed	Non-limed	Limed		
Check	Early (April 29–May 1)	192	188	190	200	770	
	Late (May 26)	124	160	206	219	710	
1—C_2H_5HgCl, 2%	Early	299	280	243	281	1103	1
	Late	187	201	226	230	844	1
2—$(C_2H_5Hg)_3PO_4$, 5%	Early	277	301	271	243	1092	2
	Late	187	190	223	213	813	4
3—$(C_2H_5Hg)_3PO_4$ + hydrated lime	Early	277	288	251	244	1060	4
	Late	186	206	193	250	835	2
4—$C_2H_5HgCl + HgCl_2$ + hydrated lime	Early	260	257	245	266	1028	6
	Late	169	198	189	204	760	8
5—$(C_2H_5Hg)_3PO_4$ + $HgCl_2$ + hydrated lime	Early	270	258	269	270	1067	3
	Late	177	213	194	207	791	6
6—C_2H_5HgCl + hydrated lime	Early	222	201	180	163	766	10[c]
	Late	144	184	183	201	712	11
7—Hydrated lime dust	Early	201	210	221	172	804	9
	Late	161	153	203	223	740	9
8—Hydrated lime wet roll	Early	199	180	181	184	744	12[c]
	Late	161	154	186	205	706	12[c]
9—Diphenyl mercury, 2%	Early	241	236	224	265	966	8
	Late	164	179	219	212	774	7
10—C_2H_5HgCl + mercury furfuramide	Early	253	263	236	232	984	7
	Late	154	204	233	238	829	3
11—Formaldehyde dust	Early	193	182	195	178	748	11[c]
	Late	149	170	211	204	734	10
12—$(C_2H_5Hg)_3PO_4$ + colloidal phosphate	Early	260	276	242	257	1035	5
	Late	174	203	217	202	796	5
Totals	Early	3144	3120	2948	2955		
	Late	2137	2415	2683	2808		

[a] Complete formula in table 7.
[b] Refer to table 8 for soil temperature and rainfall data.
[c] Less than from nontreated check.

The difference between acid and neutral soil is much more noticeable in the later count than in the one taken at the time of emergence. It can be seen that liming produced a slight increase in the number of emerged seedlings on acid soil but not on neutral soil. More seedlings survived on the limed plots, especially on acid soil. Soon after the first survival counts were made, the stand was so uniformly poor in all rows on acid soil, despite seed treatment and liming, that the entire experiment was plowed under, although, considering the rate of seeding, the planting on neutral soil would have yielded a normal crop.

Field Results, 1934. In September, 1933, enough ground limestone was added to each of three of the previously limed strips to make the total lime application 10 tons per acre, and enough sugar-factory lime cake was added to the fourth strip to make a total of 20 tons per acre. During the very dry, hot spring of 1934, wind repeatedly destroyed the sparse sugar-beet

TABLE 10.—*Number of sugar-beet seedlings emerged and surviving after 7 days from late (May 26) planting of treated and nontreated seed on limed and nonlimed acid and neutral soil.*

Seed-treatment agent[a]	Acid soil				Neutral soil			
	Nonlimed		Limed		Nonlimed		Limed	
	At emergence	7 days later	At emergence	7 days later	At emergence	7 days later	At emergence	7 days later
Check	262	65	287	120	400	341	405	371
1. C_2H_5HgCl, 2%	333	90	349	135	484	329	465	406
2. $(C_2H_5Hg)_3PO_4$, 5%	311	77	347	145	492	427	463	390
3. $(C_2H_5Hg)_3PO_4$ + hydrated lime	303	71	336	138	437	375	476	437
4. $C_2H_5HgCl + HgCl_2$ + hydrated lime	318	107	350	167	394	352	431	377
5. $(C_2H_5Hg)_3PO_4$ + $HgCl_2$ + hydrated lime	321	91	345	137	421	373	417	380
6. C_2H_5HgCl + hydrated lime	235	50	284	97	387	313	397	359
7. Hydrated lime dust	270	53	270	102	439	392	424	388
8. Hydrated lime wet roll	287	67	293	124	409	349	413	390
9. Diphenyl mercury, 2%	271	54	318	113	424	379	396	360
10. C_2H_5HgCl + mercury furfuramide	275	61	298	112	424	360	436	409
11. Formaldehyde dust	235	57	294	123	375	309	376	342
12. $(C_2H_5Hg)_3PO_4$ + colloidal phosphate	296	46	346	129	418	355	391	365
Totals	3717	889	4117	1642	5504	4714	5490	4794

[a] Complete formula in table 7.

seedling stands. These climatic factors made emergence and other counts so erratic that virtually no worth-while data were obtained. In general, the results agree with those enumerated for 1933. One set of data, however, may bear analysis.

The data above referred to resulted from an attempt to evaluate by direct macroscopic inspection of seedlings the effectiveness of seed treatment in inhibiting *Pythium de baryanum* under various field conditions.

In the 1934 plantings, which were on the same plots and similar to those made in 1933, an extra check row of 10 hills and a row of seed treated with ethyl mercuric phosphate, 5 per cent, ½ g. per 100 g. of seed, were planted on each plot to be dug up after emergence. Germination was very poor, and, since there was no apparent difference between limed and nonlimed rows, the results from both were added. An attempt was made to classify plants macroscopically as healthy, infected by *Pythium*, or by *Phoma betae.* Such classification was undertaken only after considerable practice in the greenhouse with seedlings infected with known sources of inoculum, observations of which were verified by isolation. After each of the first 2 field

readings, isolations were made to test the soundness of the field classification. A low yield of cultures was obtained, but the only *Phoma betae* observed grew from seedlings classified as infected by *Phoma betae.* Even so, a question mark is in order after the number placed in the "*Phoma*-infected" column in table 11. There seemed to be no *Phoma betae* infection on seedlings from the treated seed. The amount of Pythium infection in the early plantings also was very low in the treated rows, and not high in the nontreated rows. In later plantings a larger amount of Pythium infection occurred on seedlings from both treated and nontreated seed.

TABLE 11.—*Number of healthy and Pythium de baryanum- and Phoma betae-infected sugar beet seedlings grown on acid soil in the field from treated and nontreated seed planted at different dates, 1934*

Date planted	Date counted	Seed not treated			Seed treated		
		Healthy seedlings	Phoma-infected seedlings	Pythium-infected seedlings	Healthy seedlings	Phoma-infected seedlings	Pythium-infected seedlings
April 10	4/30	77	4[a]	5	53	0	0
" "	5/4	40	3[a]	11	54	0	0
" "	5/1	35	1[a]	4	44	0	3
" "	5/4	39	0	4	24	0	0
" 20	5/5	42	0	4	34	0	0
" "	5/5	44	0	4	57	0	1
May 10	5/15	29	3[a]	15	51	0	7
" "	5/17	44	2[a]	8	50	0	5
" "	5/21	6	0	33	47	0	18
" "	5/28	2	0	27	6	0	34
" "	5/15	43	4[a]	5	53	0	0
" "	5/17	37	1[a]	10	51	0	14
" "	5/21	12	0	31	38	0	13
" "	5/28	11	0	23	21	0	24
" 25	6/11	50	0	8	44	0	6
" "	6/14	31	0	30	29	0	35
" "	6/11	63	0	2	38	0	4
" "	6/14	18	0	31	13	0	30
June 11	6/21	34	0	21	35	0	27
" "	6/21	31	0	19	36	0	26

[a] Partially verified by isolations (see text).

Field Results, 1935. Several unexpected variables were encountered during the 1935 season. In the anticipated recurrence of severe wind damage, winter rye had been sown between each strip and each replication of the plots previously described. The rye harbored cutworms that had already appeared in the plots in 1934. They destroyed such a great number of beet seedlings, as to make it again impossible to obtain reliable data, other than emergence counts, from these plots.

Furthermore, since no crop rotation had been practiced on the plots, they had been under almost continuous fallow conditions since 1931, when a crop of corn was produced. The 1932 sugar-beet crop failed on acid soil, as did the 1933 and 1934 experimental plantings. During all this time the Pythium infestation in the surface soil apparently had been increasing in

both acid and neutral soil. Somewhat noticeable in 1934, this condition became very striking in 1935. Because of this condition and the prevalence of cutworms, the seedling stand quickly became so meager that it would have been impossible to mature a crop, even on neutral soil. The data obtained (Table 12) largely substantiate those taken in 1933.

TABLE 12.—*Number of emerged sugar-beet seedlings from 2 plantings of treated and nontreated seed on limed and nonlimed acid and neutral soil in 1935*

Seed treatment[a]	First planting (April 5; counted April 30)		Second planting (April 24; counted May 10)			
	Acid soil		Acid soil			
	Nonlimed	Limed	Nonlimed		Limed	
			At emergence	11 days later	At emergence	11 days later
Ck	74	72	28	20	31	22
1	108	139	102	77	104	85
2	126	138	100	79	106	93
3	129	116	80	57	96	81
4	122	110	89	65	123	89
5	130	129	91	66	100	73
6	134	118	49	27	47	30
Total	823	822	559	391	607	473
	Neutral soil		Neutral soil			
	Nonlimed	Limed	At emergence	11 days later	At emergence	11 days later
Total	804	794	708	578	796	694

[a] Formulae in table 14.

The numbers are total numbers of seedlings in 80 hills, from 1 seed ball in each hill. Emergence was greater in the early planting. Liming increased emergence and percentage of survival in the second planting. All seed treatments increased emergence in both plantings, with the possible exception of No. 6 (cuprous oxide) in the second planting.

TABLE 13.—*Number of seedlings from plantings on infested soil with treated and nontreated seed in 1935*

Seed treatment[a]	Average number of seedlings per 10 hills, 2 seed balls planted per hill		
	First planting May 10		Second planting May 28
	At emergence May 27	At emergence May 31	At emergence June 9
Ck	10.3	9.4	3.3
1	25.2	22.2	22.5
2	32.7	29.6	25.9
3	28.1	25.6	20.0
4	26.1	24.6	20.0
5	26.7	24.2	23.1
6	24.6	22.0	9.1

[a] Formulae in table 14.

The data in table 13 were taken from a similar planting on a different plot, also fallowed for one year and almost as heavily infested. The numbers represent average number of seedlings per 10 hills, from 2 seed balls per hill. Here, again, there was greater mergence in the early planting and from treated seed. Cutworm injury made it necessary to abandon this experiment also. In fact, it was necessary to uncover the stubs of damaged seedlings in order to make the emergence counts for the second date of planting.

The seed treatments used in 1935 are listed in table 14.

TABLE 14.—*Sugar-beet seed treatments used in 1935*[a]

Treatment	Toxic agent	Rate of application
1	Ethyl mercuric phosphate, 5 per cent	¼ g. per 100 g. of seed
2	Ethyl mercuric phosphate, 5 per cent	½ g. per 100 g. of seed
3	Combination: Ethyl mercuric phosphate, 5 per cent 25 g. Mercury furfuramide, 5 per cent 75 g.	1 g. per 100 g. of seed
4	Combination: Ethyl mercuric phosphate, 5 per cent 25 g. Merko (3½ per cent metallic mercury) 75 g.	1 g. per 100 g. of seed
5	Combination: Ethyl mercuric phosphate, 5 per cent 50 g. Talc filler 50 g.	1 g. per 100 g. of seed
6	Cuprous oxide	1 g. per 100 g. of seed

[a] See tables 12 and 13.

DISCUSSION

Previous to this study, no one in America had definitely shown that a single pathogen has been the principal cause of damping off of sugar beets in any given locality over a period of years. Peters (19), after his investigations in Germany, clearly indicated that he considered *Pythium de baryanum* the chief cause in what were probably some acid German soils in which the situation was similar to those on which stand failures occur in northern Iowa. Edson (7) included 4 pathogens, *P. de baryanum, P. aphanidermatum, Phoma betae,* and *Rhizoctonia* sp. as causative agents, but indicates no predominating parasite. It is significant, however, that, when he isolated from beet seedlings grown on Wisconsin soil, his cultures were chiefly *Pythium,* while from seedlings grown on Kansas and Colorado soils he cultured only *Rhizoctonia*; and, as he says of plantings in these latter soils, "A good stand—was secured—in spite of damping-off." Cultures of *Rhizoctonia* have been rarely isolated from diseased beet seedlings from

areas of stand failures in northern Iowa. The writer is not aware that *Rhizoctonia* has destroyed a seedling stand of sugar beets in northern Iowa to the extent that replanting was necessary.

Coons and Stewart (5) also made no attempt to single out one pathogen as consistently more important in causing seedling stand failures, but they did try to differentiate the symptoms brought about by infection with *Pythium, Phoma betae,* and *Rhizoctonia.*

The restriction of severe damage to localized areas within fields leaves little doubt that the pathogen is soil-borne, and almost certainly eliminates *Phoma betae* as a possible causative agent. Seedlings grown in steamed soil from treated *Phoma betae*-infested seed lots have been nearly disease-free. Evidence also has been presented indicating control of seed-borne *Phoma betae* in the field by seed treatment. On the other hand, seed treatment has by no means guaranteed a good stand from late plantings, although it should have been had the principal infective agent been seed-borne *Phoma betae.*

In the writer's experiments, *Pythium de baryanum* has been a much more active parasite than *Phoma betae.* Seed-borne *P. betae* brought about some noticeable injury in steamed soil in the greenhouse if the seedlings were allowed to grow for 2 weeks or more after emergence. *Pythium de baryanum,* in naturally infested field soil, destroyed many seedlings from the same lot before they emerged and many more within a week after emergence. Isolations from field-grown seedlings in early stages of infection and invasion have in most cases yielded cultures of *Pythium,* most frequently *P. de baryanum.* *Phoma betae* has been recovered occasionally from seedlings grown in the field under low-temperature conditions unfavorable to *Pythium.* *Rhizoctonia* rarely has been recovered from seedlings grown on areas where stands fail.

Recommendations for control of seedling diseases of sugar beet have thus far been lacking or somewhat indefinite. Arrhenius (1), in addition to noting the abundance of the disease on acid soils, recognized the importance of *Pythium de baryanum* in late plantings. He reports control by liming, but apparently made no date of planting experiments. Edson (7) also records circumstantial observations of severe damage by *Pythium de baryanum* in late plantings in Wisconsin, but, likewise, made no date of planting experiments. Coons and Stewart (5) were concerned primarily with seed treatment as a control measure, but considered their field results were insufficiently consistent to warrant recommendations. In 1937 LeClerg (15) reported his results with Ceresan. At the same time, Reddy and Buchholtz (20), on the basis of the work herein presented, outlined the procedures necessary to improve seedling stands in northern Iowa, namely: plant early with seed treated with 5 per cent ethyl mercuric phosphate; if late planting is imperative, avoid acid, heavily infested fields or areas.

The failure of *Pythium de baryanum* to grow and induce infection rapidly at temperatures not too low for beet-seed germination and seedling

development is a part of the disease picture that is vulnerable to control measures. At soil temperatures below 15° C., Pythium apparently is inactive enough for an adequate seed treatment to check it until the seedlings are too large to be invaded, or, if infection takes place, the invasion is checked before it threatens the life of the young seedling. In 3 successive years (1933–1935) the first plantings of seed treated with 5 per cent ethyl mercuric phosphate produced better stands than any from treated or nontreated seed planted thereafter. Late plantings on acid, heavily infested soil have failed in spite of seed treatment. Generally, any planting made after the first week in May would be a late planting in northern Iowa.

Continued heavy lime dressings have, in time, brought about slight increases in seedling emergence, but not so large as those resulting from earlier planting and seed treatment. The most noticeable effect of lime, even in smaller quantities, has been the improvement in the percentage of survival of emerged seedlings, although in later plantings the effect has not been sufficient to produce a satisfactory stand. Liming by itself has not resulted in sufficient improvement to warrant its expense, but of course, if liming were an established part of a grower's farm program, the effect would be favorable both to stand and yield.

One control practice that has been demonstrated by Coons and Kotila (6), namely, planting on corn land rather than after alfalfa or sweet clover, has been adopted by beet growers in Michigan, but it has not yet produced similar effects for the writer in preliminary experiments in northern Iowa.

SUMMARY

Damping off induces sugar-beet stand failures in northern Iowa under conditions of soil acidity and moderately high soil temperature.

Pythium de baryanum, Phoma betae and *Rhizoctonia* are pathogenic to sugar-beet seedlings in the greenhouse. In the field, *Pythium de baryanum* causes over 95 per cent of the damage to germinating and emerging seedlings.

Pythium de baryanum causes a general necrosis of any part of the germinating and emerging seedling and cortical necrosis on slightly larger plants. *Phoma betae* causes a slow cortical necrosis that frequently results in no aboveground symptoms and from which seedlings may recover in field soil. Only a very small percentage of diseased seedlings grown in the field yielded cultures of *Phoma betae*. *Rhizoctonia* has very seldom been isolated from field-grown sugar-beet seedlings.

The *Pythium* isolates taken from sugar-beet seedlings are similar, but not identical. The measurements of their fruiting structures are as follows: Conidia, 10 to 35 μ; oogonia, 16.6 to 22.1 μ; oospheres and oospores, 13.6 to 17.0 μ. Their measurements and cultural characters are like those listed by Butler for *Pythium de baryanum.*

Temperatures below 15° C. were unfavorable to the growth of *Pythium de baryanum* in pure culture and its pathogenicity to sugar-beet seedlings.

Areas in which *Pythium de baryanum* brought about especially poor stands did not differ greatly from areas with good stands in moisture content, but were found generally to be more acid, *e.g.*, below a pH of 6.5. In 1933, a survey of sugar-beet fields in northern Iowa revealed a correlation of 0.745 between soil pH and percentage stand. Two cultures of *Pythium de baryanum* grew uniformly well at pH values of 4.7 to 7.3.

In field experiments liming increased the number of emerging seedlings only slightly, but increased the percentage of survival. Early planting increased emergence and survival. Seed treatment also increased emergence and survival, and was most effective in early plantings.

No single practice has completely controlled damping off of sugar beets. A combination of early planting and seed treatment (5 per cent ethyl mercuric phosphate, 4–7 oz. per 100 lb. seed) can be recommended to improve stands of sugar beets.

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INFECTION OF MAIZE WITH PHYTOMONAS FLACCUMFACIENS, P. INSIDIOSA, P. MICHIGANENSIS, P. CAMPESTRIS, P. PANICI AND P. STRIAFACIENS[1]

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INTRODUCTION

According to Burkholder (1) species of the genus Phytomonas may be divided into several natural groups on the basis of cutural characteristics. One group composed of *P.*[2] *stewarti* (E.F.S.) Bergey *et al.*, *P. flaccumfaciens* (Hedges) Bergey *et al.*, *P. insidiosa* (McCulloch) Bergey *et al.*, *P. michiganensis* (E.F.S.) Bergey *et al.*, and possibly several others has been referred to as the *Stewarti* group, from one of its earliest described species. Another group perhaps not so well defined has been referred to as the *Campestre* group. Remaining species are colorless and may be grouped together on this basis.

Hosts attacked by the organisms of any one group may be widely different. For example, in the *Stewarti* group, *Phytomonas stewarti* primarily causes a wilt of maize (*Zea mays* L.), *P. flaccumfaciens* a wilt of beans (*Phaseolus vulgaris* L.), *P. insidiosa* a wilt of alfalfa (*Medicago sativa* L.), and *P. michiganensis* a wilt of tomatoes (*Lycopersicum esculentum* Mill.). Since these organisms are very similar in cultural behavior, one might expect that a strain of maize very susceptible to *P. stewarti* may also be infected to some extent at least by other organisms of this group or, *vice versa*, *P. stewarti* may infect alfalfa, beans, and tomatoes. This paper is chiefly concerned with such cross-inoculation studies. Effect on maize of *P. flaccumfaciens*, *P. insidiosa*, and *P. michiganensis* of the *Stewarti* group, *P. campestris* (Pam.) Bergey *et al.* of the *Campestre* group, and such colorless forms as *P. panici* (Elliott) Bergey *et al.* causing bacterial stripe of proso millet, *Panicum miliaceum* L., (2) and *P. striafaciens* (Elliott) Bergey *et al.* causing bacterial stripe of oats, *Avena sativa* L., (3) is described. Effect of *P. stewarti* on hosts of certain of the above organisms also is described.

[1] This is part of the research carried on in the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research, Princeton, New Jersey, while on a General Education Board Fellowship.

[2] The abbreviation *P.* instead of *Phyt.* is used for *Phytomonas* to conserve space.

MATERIALS AND METHODS

Cultures of *Phytomonas insidiosa, P. michiganensis,* and *P. campestris* were obtained from George L. McNew, Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research. Degree of pathogenicity of *P. insidiosa* for alfalfa was not determined. The cultures of *P. michiganensis* and *P. campestris* were found to be fairly pathogenic on Bonny Best tomatoes and Early Jersey Wakefield cabbage, *Brassica oleracea* var. *capitata,* respectively. A culture of *P. flaccumfaciens* was obtained from Florence Hedges, U. S. Dept. Agr., and was found very pathogenic for both Scotia and Golden Cluster beans. Cultures of *P. panici* and *P. striafaciens* were obtained from Charlotte Elliott, U. S. Dept. Agr. These were old stock cultures and proved to be weakly pathogenic for proso millet and Early Pearl oats, respectively.

Two inbred lines of maize were used in testing maize infection with the above organisms. One, GB797, was an inbred line of Golden Bantam sweet corn very susceptible to *Phytomonas stewarti;* the other, OSF, was an inbred line of field corn, very resistant to *P. stewarti.* A detailed description of the genetic resistance or susceptibility of these lines of *P. stewarti* was given in an earlier paper (6). Organisms to be tested on maize were hypodermically injected into week-old seedlings of these two lines. Two punctures per seedling were made: one, at the first node above the mesocotyl; the other, through the leaf sheaths just above the growing point. This method was similar to that used in testing resistance and susceptibility of various inbred lines to *P. stewarti* (6). All tests were made in a greenhouse. Seedlings were grown in 4-inch pots filled with fertile soil, and amply watered. Temperatures were maintained at 80°–90° F. Each test was repeated several times.

To test infection of beans, tomatoes, cabbage, millet, and oats with a strain of *Phytomonas stewarti* highly pathogenic for maize, the following varieties were used: Golden Cluster beans, Bonny Best tomatoes, Early Jersey Wakefield cabbage, proso millet, and Early Pearl oats. These varieties proved to be susceptible to *P. flaccumfaciens, P. michiganensis, P. campestris, P. panici,* and *P. striafaciens,* respectively. Tomato and cabbage plants were started in flats and later transplanted to 6-inch pots. After transplanted plants had become established, inoculations were made by puncturing stems, petioles, and the veins of leaves with a hypodermic needle. Beans were similarly inoculated shortly after emergence. Oats and proso millet were inoculated in seedling stage by a single puncture at the base of the stem.

OBSERVATIONS

Effect of Vascular Parasites of Beans, Alfalfa, Tomatoes, etc., on Maize

Phytomonas flaccumfaciens: First indication of infection of the maize strain, GB797, appeared about 4 days after inoculation as a yellowing of

certain veins of the leaves and a slight discoloration of the parenchymatous tissue on either side. Discoloration of tissue along affected veins was very irregular. Dull-grey areas often appeared intermingled with light and dark green. Brown water-soaked lesions along affected veins, characteristic of infections with *P. stewarti,* did not appear. Very often, however, affected veins became transparent. Later the leaves would split along such veins.

On most seedlings, infection was not severe enough to kill the plants, but, invariably, some of the leaves, unfolded at the time of inoculation, were killed. Often one longitudinal half of a leaf died while the other half remained green. Sometimes, the growing point was killed and the original plant succumbed. Usually, however, such plants developed suckers, which generally were not affected. All infected plants were severely dwarfed.

Microscopic examination of diseased plants showed many of the xylem vessels of the stem, leaves, and mesocotyl filled with bacteria and bacterial slime.

Infection on the strain of maize, OSF, was less severe than on GB797. A few affected veins of the leaf were evident, but dwarfing and mutilation of the plant in general was much less severe (Fig. 1, A and B).

Phytomonas insidiosa: Both OSF and GB797 were affected by this organism, but OSF seemed to be more severely attacked than GB797. Symptoms of infection on the leaves of either host were not numerous. Chlorotic streaks along affected veins occasionally appeared. On one of the inoculated plants of OSF, water-soaked areas of irregular size and shape were noted along one of the affected veins of a leaf.

A common sign of infection of OSF was the drying up of the parenchymatous tissue surrounding the wound made by the inoculating needle through the leaf sheaths just above the growing point on very young seedlings. The plants would often become girdled in this region by a band of dead or dying parenchyma tissues 3–5 mm. wide, and parts above would topple over. Apparently the vascular bundles running through the band of dead or dying parenchyma on the sheath were not seriously affected, for the leaf above or sheath below the affected spot remained normal for a considerable period.

Undisputable signs of infection of OSF were evident upon microscopic examination of the vascular tissues. Figure 2, A, shows a cross section of the mesocotyl of an infected OSF seedling about 1½ inches below the point of inoculation. It may be seen that a considerable number of the vascular elements were plugged or partially plugged. Bacteria could be found in certain vessels of the mesocotyl all the way down to the point of seed attachment. They also were found in certain vessels of the stem, leaves, and leaf sheaths. Figure 2, B, shows a vascular bundle in a leaf sheath in which all the xylem vessels were plugged with a yellow gum-like mass.

Phytomonas michiganensis: GB797 was more readily attacked than OSF. Symptoms of infection on either host were very similar to those obtained with *P. flaccumfaciens* but somewhat less severe. Affected seedlings usually

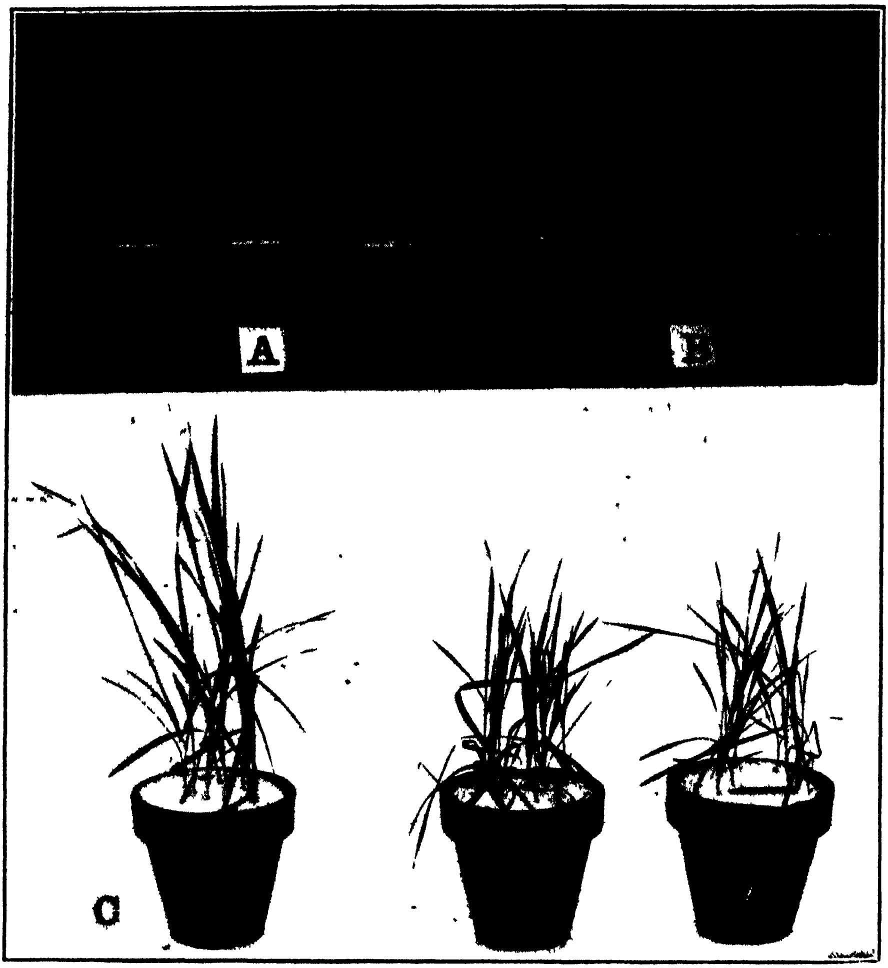

FIG. 1. Effect of *P. flaccumfaciens* on the inbred lines of corn, OSF and GB797. A. OSF, pot on left, control. B. GB797, pot on right, control. Photographed 10 days after inoculation. C. Effect of *P. stewarti* on Early Pearl oats. Pot on left, control. Photographed 15 days after inoculation.

were dwarfed. The growing point on some was killed, but on such plants suckers readily developed.

Upon microscopic examination of the vascular tissue, many of the vessels throughout the mesocotyl and certain bundles of the stem and leaves were found either completely or partially plugged. Reisolations made from infected veins of leaves were tested on tomatoes. Symptoms of tomato wilt appeared.

Phytomonas campestris: This organism was very reluctant in attacking maize. On OSF no infection was detected. On GB797, the outward signs of infection were manifest largely as dwarfing of the plants. Occasionally, affected veins could be detected on the leaves by a slight discoloration, but such signs were comparatively rare.

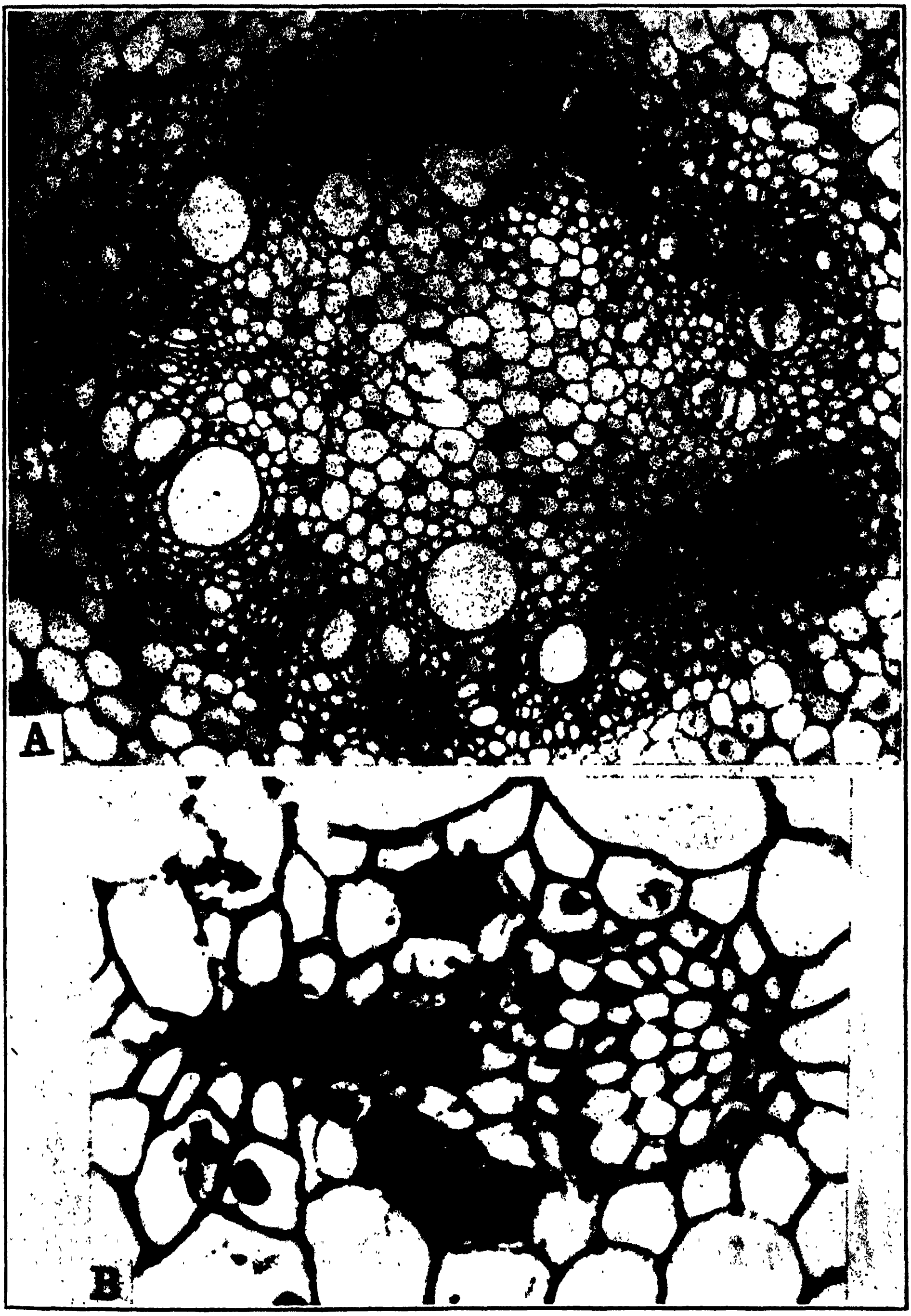

FIG. 2. A. Cross section of the mesocotyl of an infected OSF seedling inoculated with *P. insidiosa*. The yellow gum like masses of bacteria and slime appear black in the photograph. B. Cross section of an infected vascular bundle in a leaf sheath of an OSF plant inoculated with *P. insidiosa*. The xylem elements (dense black areas) were completely plugged.

Upon microscopic examination of the vascular tissues of dwarfed plants a few affected vessels of the mesocotyl and stem were evident. Reisolations made from affected vessels of the stem readily infected and wilted plants of Early Jersey Wakefield cabbage.

Phytomonas panici: Symptoms induced on GB797 by *P. panici* were a little more severe than those produced by *P. campestris.* A slight discoloration of certain veins of the leaves was evident 4–5 days after inoculation. Many of them were bordered on each side by a narrow irregular band of light-green parenchyma. Later, a few small necrotic areas often appeared at irregular intervals along affected veins. Plants were often severely dwarfed. On OSF no infection was detected. Reisolations were made from affected veins of GB797 plants and tested on proso millet. A mild degree of infection, similar to that caused by the original culture, was obtained.

Phytomonas striafaciens: Effect of *P. striafaciens* on GB797 was similar to that of *P. campestris.* The plants were only slightly dwarfed. An occasional vein in the leaves showed a slight discoloration. No signs of infection on OSF were apparent.

Effect of Phytomonas stewarti on Beans, Tomatoes, Cabbage, Proso Millet, and Oats

Fifteen plants of Golden Cluster beans were inoculated with a strain of *Phytomonas stewarti* highly virulent for maize. No outward signs of infection, with the exception of a slight stunting, was evident. Upon examination of the stem tissues four weeks after inoculation, discolored nodes and inter-nodal vessels were found. Reisolations were made from the inter-nodal discolored vessels and their pathogenicity was tested on maize. No change in virulence for maize was noted.

Six plants each of Bonny Best tomatoes and Early Jersey Wakefield cabbage, that were about 3 weeks old, were inoculated with *Phytomonas stewarti.* No infection was evident. The extent of these trials, however, does not justify the conclusion that the plants are immune.

Proso millet was readily infected with *Phytomonas stewarti,* as pointed out previously by Ivanoff (4) and Wellhausen (7). Numerous water-soaked lesions appeared along the veins of the leaves; resembling in manner of development those produced by *P. stewarti* on maize (5). Plants were severely dwarfed but none were killed.

Early Pearl oats also was readily infected with *Phytomonas stewarti.* Lesions on the leaves resembled very closely those produced by this organism on maize. Well-defined semicircular, water-soaked areas appeared along affected veins of leaves 3–4 days after inoculation. These areas often coalesced, forming long, narrow, water-soaked stripes 1–2 mm. wide along the veins. The stripes in time became necrotic and turned light brown in color. Infected plants were usually severely dwarfed but not killed. Figure 1, C, shows a number of oat plants 15 days after inoculation beside controls. Dwarfing of the inoculated plants is evident.

In addition to the above, a variety of soft red winter wheat, thought to be Leap, was tested with *Phytomonas stewarti*. A few water-soaked lesions appeared on the leaves, but, in general, this wheat variety was much less susceptible to *P. stewarti* than was Early Pearl oats.

DISCUSSION AND CONCLUSIONS

In these experiments maize was inoculated with the various organisms when plants were very young and growing rapidly under conditions of high temperature, ample moisture, and fertile soil. It may be argued that, under such conditions, seedlings are very susceptible to mechanical injury and that much of the yellowing and dwarfing observed may have been induced by mechanical injury in the process of inoculation. This possibility, however, may be ruled out, since injury caused by needle punctures in the process of inoculation seemed to have very little if any noticeable effect on the development of the plant as apparent from adequate checks. The yellowing and dwarfing were largely due to the presence of the organism.

It was apparent throughout these investigations that certain of the organisms, namely, *Phytomonas flaccumfaciens*, *P. insidiosa*, and *P. michiganensis*, grew or multiplied more rapidly in the medium of the corn plant than *P. campestris*, *P. panici*, and *P. striafaciens*. The first 3 organisms mentioned usually caused considerable injury and often killed the young plants. The last 3 seemed to affect the host very mildly, growing and spreading very slowly at first, but apparently growth soon ceased. It is entirely possible that the mild symptoms brought about by the latter 3 organisms were due not to any special affinity or adaptation on the part of the organism for the host, but to the fact that these young, rapidly growing plants were full of sap and, temporarily, may have contained constituents conducive to a small amount of growth. It is possible that a wide range of unrelated organisms would cause a similar slight dwarfing and yellowing of certain veins of highly turgid seedling plants under optimum conditions for rapid growth.

With *Phytomonas flaccumfaciens*, *P. insidiosa* and *P. michiganensis*, the situation seemed to be somewhat different. These organisms apparently possessed a greater affinity or were better adapted to the medium of the xylem vessels. This is not surprising, since these organisms appear to be more similar to *P. stewarti* in cultural characteristics than the others.

From the results obtained, it is not contended that corn may be a natural host of *Phytomonas flaccumfaciens*, *P. michiganensis*, etc., or, for example, that beans may be a natural host of *P. stewarti*, in the sense that these organisms would naturally cross-inoculate or maintain themselves indefinitely on these hosts under field conditions. The results merely indicate that, of the organisms tried, those closely related to *P. stewarti* in cultural characteristics, as determined from artificial media, are also capable of considerable growth in the medium of the corn plant under conditions most favorable to *P. stewarti*. Whether the pathogenicity for maize of *P. flaccumfaciens*, *P. michiganensis*, or some of the others may be increased by repeated passage

through maize, or whether the pathogenicity of *P. stewarti* for oats or some of the other plants may be increased by successive passages through certain strains of these plants remains to be determined. Studies on these points are under way.

SUMMARY

Effect of *Phytomonas flaccumfaciens, P. insidiosa, P. michiganensis, P. campestris, P. panici* and *P. striafaciens* on maize seedlings, and effect of *P. stewarti* on certain hosts of these organisms is described.

P. flaccumfaciens, P. insidiosa and *P. michiganensis* usually severely injured and often killed inoculated maize seedlings. The others had a more mild effect. Two inbred lines of maize were used in these tests, one (GB797) was very susceptible to *P. stewarti*, the other (OSF) was very resistant to *P. stewarti.* With one exception (*P. insidiosa*), all the organisms tried attacked the line, GB797, more readily than the line, OSF. *P. insidiosa* affected OSF more readily than GB797.

Types of symptoms induced on maize were, in general, very similar. Infection was primarily confined to the xylem elements. Plugging of the xylem vessels of certain vascular bundles in leaves, stems, and mesocotyls of affected seedlings was apparent in varying degree upon microscopic examination several weeks after inoculation. Affected veins in the leaf and tissues adjoining such veins were usually discolored. Very often tissues along affected veins became transparent.

Golden Cluster beans inoculated with *P. stewarti* were slightly dwarfed and showed some discoloration of the nodal and inter-nodal vessels of the stem.

No infection was evident on Bonny Best tomatoes and Early Jersey Wakefield cabbage inoculated with *P. stewarti.*

Proso millet and Early Pearl oats were readily infected by *P. stewarti.* Brown water-soaked irregular stripes, similar to those produced by *P. stewarti* on maize, developed along affected veins.

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STUDIES OF BLACK ROOT ROT OF APPLE[1]

F. D. FROMME AND F. J. SCHNEIDERHAN[2]

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The studies reported herein are a part of a general study of the black root rot of apple, caused by *Xylaria mali* Fromme, in progress at the West Virginia Station, and are a continuation of studies previously conducted at the Virginia Station by the senior author and others.[3]

I. INFECTION OF STOCKS THROUGH EXPOSURE TO INFESTED SOIL AND DISEASED ROOTS

A study of the invasion of apple roots by *Xylaria mali* under conditions of natural exposure to inoculum in infested soils and diseased roots was made in 2 orchards in Jefferson County, West Virginia. Selected rootstocks were employed and, although none of them exhibited any evidence of resistance, the resultant incidence and intensity of infection are of considerable interest.

The 165 2-year-old trees used in the study were representative of 45 clons that had been propagated from root cuttings of selected seedlings. The seedlings represented 20 open-pollinated varieties of the cultivated apple, *Malus malus*, 15 of American and 5 of European and Asiatic origin; 3 clons of *M. malus* developed by Guy E. Yerkes of the U. S. Department of Agriculture, and 1 clon of *M. zumi*, also originated by Yerkes. The number of individual trees per clon varied from 1 to 20.

The trees were pruned to single whips averaging about 30 inches in height and were set on November 25, 1932, in locations where apple trees had died of black root rot. Areas 10 by 10 feet square were prepared by spading and raking and the trees were set in rows 2 feet apart and were spaced 1 foot apart in the row. All old roots were removed to the depth of the spade. The land was Hagerstown loam, representative of the best of the orchard soils of the county. Four separate locations were used, 3 in the Border orchard, near Kearneysville, and one in the Dutrow orchard, near Charles Town. Each planting will be discussed separately.

Border Orchard

The orchard was about 25 years old, and a number of the original trees had died or were in poor condition because of root rot. Many of the latter showed typical symptoms of black root rot, and occasional stromata of *Xylaria mali* were present.

Site No. 1. The original tree had been removed and was represented by a 4-year-old replant that had died of root rot. All roots, except one, had

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2 Acknowledgment is made of the services of Dr. L. H. Leonian, who made many of the isolations and furnished cultures for inoculation work.

3 Fromme, F. D., The black rootrot disease of apple. Va. Agr. Exp. Sta. Tech. Bull. 34. 1928.

been killed and showed typical Xylaria infection. These were removed, and, since no remnants of the roots of the original tree were found, the test on this site was one of infested soil lacking diseased root material other than that that had thoroughly disintegrated.

Forty-six trees were set in this site and, except for one that had died of root rot in 1934, all were alive after 3 seasons' growth. All remaining trees were dug carefully on November 22, 1935, and the dirt was washed from the roots with a hose. Including the tree that had died in 1934, a total of 21 trees showed typical Xylaria root infections, while 25 showed no infection, an infection percentage of 45.7. In some cases infection had been established on the main root; in others main laterals or smaller roots had been invaded, and a number of trees showed 3 or 4 separate lesions. Those with

FIG. 1. Black root rot lesions on the main root and crown of a 2-year old apple tree from Site No. 2. The lesions are indicated by arrows, and the brittle character of the affected wood is shown by the broken tip of the root. Photograph by Dr. A. B. Groves.

severe invasion of the main root and crown doubtless would have died before completion of another season's growth. Typical examples of infection, such as occurred in this and the other plantings, are shown in figures 1 and 2.

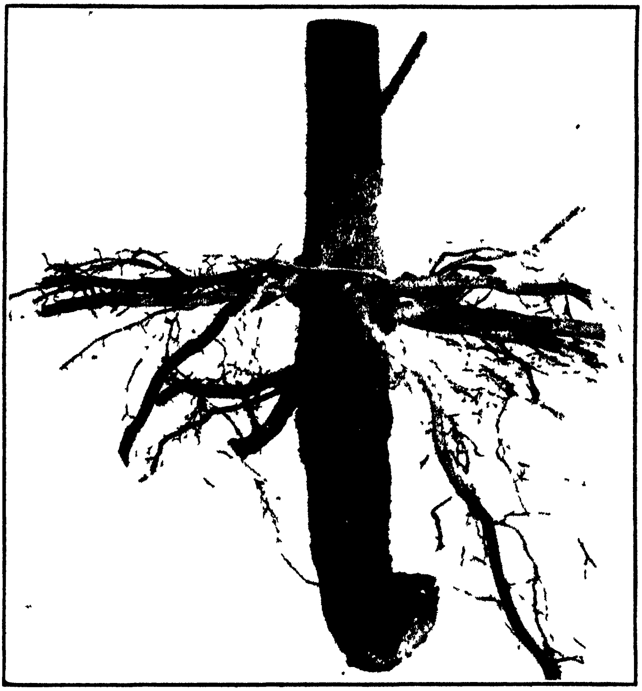

FIG. 2. A 3-year-old tree of *Malus zumi* from Site No. 3. The base of the main root has been completely invaded, and the tree was supported by adventitious roots thrown out above the affected zone. The main root broke off in harvesting.

One of the interesting features of this planting was the localization of the infected trees with reference to the position of the replant and original tree. With one exception all of the infections occurred on one side of this position (Fig. 3). Cases of infection of only one side of the root system of mature trees are rather common, and it would appear in this instance that the original tree had such a one-sided infection and that the fungus persisted in this area, but was not present in appreciable degree in the soil of the other side. Since the root system of the 4-year-old replant was not extensive enough to invade all of the infested area, it also appears that the fungus had persisted there for at least 4 years in the absence of growing apple roots.

Xylaria mali was isolated readily from specimens of root lesions taken from each of the 4 sites included in the study.

Site No. 2. The original tree had been removed in the spring of 1932. The stump, left near the site, and many of the roots remaining in the soil showed typical Xylaria rot. All large roots were removed, chopped into small pieces, scattered over the bed, and worked into the soil with the spade and rake before the young trees were set.

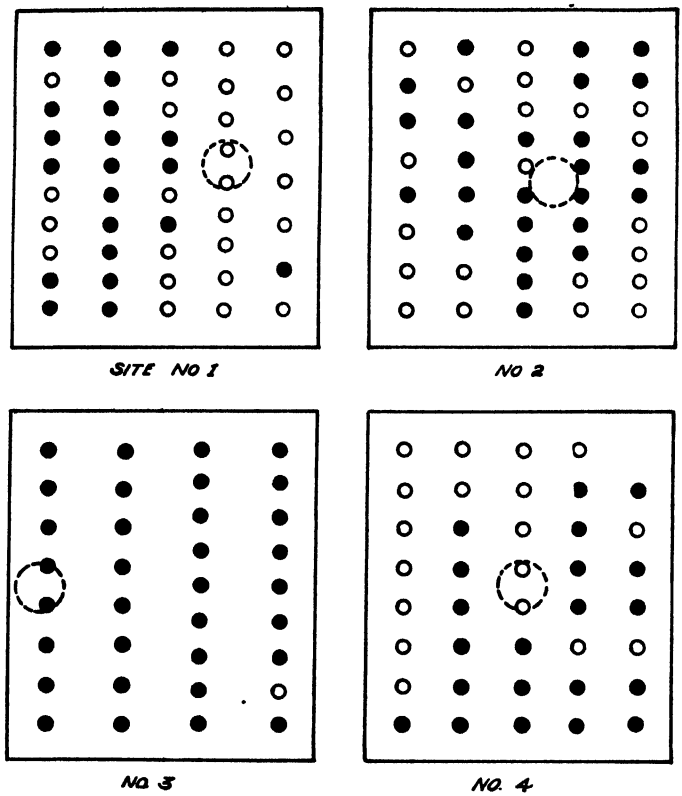

FIG. 3. Diagrams showing location of infected (black circle) and noninfected (open circle) apple trees in 4 experimental plantings. The broken circles show approximate location of the original trees.

The 46 young trees used in this site were all living after 2 seasons' growth. They were harvested November 16, 1934. Twenty-five, or 54.3 per cent, showed Xylaria infection, while 21 were not infected. Of those infected, 11 showed extensive injury, which would seemingly have resulted in death within another year. The main roots were affected, the lesions varying from 2 to 6 inches in length, and the crowns completely girdled. The other 14 trees showed infections on lateral roots only, some bearing only a single lesion, while others bore 3 or 4 separate lesions.

The location of infected and noninfected trees, as shown in figure 3, indicates a more intense infestation in the area adjacent to the center of the original tree than at the borders, and suggests that soil infestation was of greater importance than the scattering of diseased roots.

Site No. 3. This was similar to Site No. 2 in that the original tree had been removed in the spring. The stump left nearby showed that death had followed Xylaria infection. The roots remaining in the soil indicated that infection had occurred chiefly on one side of the root system. The young trees were, therefore, set in this infested area. Affected roots were chopped in small pieces scattered over the area and worked into the ground.

In all, 34 young trees were set and, although all were living after 3 seasons' growth, many were noticeably dwarfed and showed very severe Xylaria infection when harvested on December 4, 1935. Only one tree of the lot showed no infection. Extensive invasion of the main root and crown, as well as some infections of lateral roots, had occurred in 20 trees. The other 13 showed infections on lateral roots only. Ten or more separate lesions were observed in a single root system in some instances. Since all but one of the trees showed infection, no pattern of distribution of the inoculum was apparent. The planting is of particular interest because of the very high (97.1 per cent) incidence of infection.

Dutrow Orchard

Site No. 4. The original trees in this orchard were approximately 30 years old, and a considerable number were missing or were represented by replants of various ages. The young trees were set on the site of an original tree that had been removed a few months earlier but had been left lying at the site. The root system was almost completely invaded by *Xylaria mali* and the organism was readily obtained in pure culture. The old roots were removed from the soil, chopped fine, and distributed in furrows before the young trees were set.

All trees were living at the time of harvest, November 5, 1935. Of the 39 trees, 21, 53.8 per cent, were infected and 18, or 46.2 per cent, showed no infection. Nine of these showed infection on the main root or crown; the others, on main laterals or small roots. One tree was almost dead, the crown having been completely girdled by the fungus.

The diagrammatic representation of this planting shows no evident pattern of distribution of the inoculum.

Although it is known that young apple trees rarely survive to bearing age when set as replants following trees that have been killed by the black rootrot fungus, the plantings reported here supplement this information and reveal the extent and intensity of infection that may occur within 2 and 3 years on young trees planted in thoroughly infested soil.

A summary of the data from the 4 plantings (Table 1) shows that the incidence of infection ranged in the separate plantings from 45.7 to 97.1 per cent and averaged 60.6 per cent. Because of the difficulty involved in har-

Table 1.—*Incidence of infection by Xylaria mali in young apple trees set November 25, 1932, in infested soil*

Site	Period of exposure	Number infected	Number not infected	Percentage infected
1	3 years	21	25	45.7
2	2 years	25	21	54.3
3	3 years	33	1	97.1
4	3 years	21	18	53.8
All		100	65	60.6

vesting and examining complete root systems in the heavy soils employed in the tests, it is probable that a number of lesions on small roots were overlooked and that higher incidences of infection actually obtained.

The intensity of infection varied from lesions on small roots of the current year to extensive invasion of the main root and crown. Many individuals exhibited more than one lesion and in some as many as 10 separate lesions were recorded. Although death of the tree resulted in only one instance, many of the trees were so seriously affected that they would scarcely have survived another season.

The pattern of disease indicated that infection commonly occurs on newly developed roots and progresses from these into older roots and, eventually, into the crown. Death, which results from the complete girdling of the crown, may be delayed by the production of adventitious roots above the affected area. There was no evidence of any toxic effect on the plant as a whole.

While the relative importance of soil and affected roots as sources of inoculum cannot exactly be defined, from these studies, it is evident that soils may carry inoculum in the absence of visible root material.

No evidence of any promising measure of resistance was found among the 44 clons of *Malus malus* or the single clon of *M. zumi*. Absence of infestion is attributed to lack of contact with inoculum rather than to disease resistance.

II. SUSCEPTIBILITY OF STOCKS TO PURE CULTURE INOCULATION

In addition to the plantings in infested soil, inoculation tests with *Xylaria mali* in pure culture were made on a group of 23 selected rootstocks of *Malus*. All of these are included in stock-scion studies now in progress at the University Experiment Farm, Kearneysville, West Virginia, the studies being cooperative between the West Virginia Agricultural Experiment Station and the Division of Horticultural Crops and Diseases of the Bureau of Plant Industry, U. S. Department of Agriculture.

The trees used in our tests were remnants from the stock-scion planting and included 12 clonal stocks, 5 originated by the East Malling Research Station, and 7 by the United States Department of Agriculture, and 11 seedling stocks of open-pollinated varieties. The stocks had been budded to the varieties Gallia, Jonathan, Red Rome, Starking, Staymared, and York; but, as there was no evidence of a scion influence on their susceptibility, they will be treated in this report as though a single scion variety had been used. All material, except East Malling stocks 1, 3, and 13, was supplied by the cooperating federal agency. The exceptions were grown and budded by the Department of Horticulture of the West Virginia Station.

A total of 465 trees, of vigorous 1-year whips on 2- or 3-year roots, were inoculated with a mixture of 8 isolants of *Xylaria mali*, grown in pure culture on steamed rice, and planted on March 31, 1933, in Hagerstown loam at the Kearneysville experiment farm. They were spaced 30 inches apart

in rows 12 feet apart. The inoculum was applied as a plaster on a small area of a main lateral root from which the bark had been scraped, and was held in position with a narrow band of cloth.

The soil was quite dry when the trees were set, and a rather prolonged period without rainfall ensued. These conditions were evidently unfavorable for infection, since only 26 of the 465 inoculated trees became infected. Five of these died after 2 seasons of growth and the others were living when harvested in November, 1935. Twenty-seven other trees died from causes other than black root rot.

The inoculation was repeated on June 7, 1935, with a pure culture of *Xylaria mali*, designated as 1–I in our series and isolated from a diseased York Imperial apple tree, near Kearneysville. A total of 86 apple trees of the planting used in the preceding test, now 2 years old, were inoculated *in situ*. The soil was removed until a side root, of about the diameter of a pencil, was exposed. This was scraped lightly with a knife, the inoculum applied, and the soil replaced. Only vigorous, healthy trees were employed. Soil conditions at the time and for some weeks thereafter were excellent for the establishment of infection, as there was abundant rainfall.

TABLE 2.—*Susceptibility of rootstocks of Malus to Xylaria mali. The figures in parentheses show numbers of infected trees that died of root rot*

Clonal and seedling stocks	Inoculations of 3–31–1933 Number		Inoculations of 6–7–1935 Number	
	Inoculated	Infected	Inoculated	Infected
Clonal stocks				
East Malling, Type 1	23	3	7	7
" 2	27	1	3	3
" 3	8	0	3	3
" 13	17	0	3	1
" 15	12	1	3	3
U.S.D.A. Type 200	4	0	1	1
Vermont 313	19	0	5	5
316	42	5(1)	3	3
317	18	1(1)	3	3
323	21	2	3	3
Minnesota 329	60	7(1)	3	3
Malus zumi	4	2(2)	2	2
Seedlings stocks				
Delicious	29	0	6	6
Fameuse	24	0	6	6
French Crab	14	0	3	3
Grimes	9	0	3	3
Jonathan	7	0	3	3
McIntosh	37	2	7	6
Northern Spy	31	1	7	5
Rome	19	1	4	4
Tolman	9	0	3	3
Wealthy	14	0	5	5
Winesap	17	0	5	5
Total	465	26(5)	91	86
Per cent		5.6		95.5

The trees were harvested 171 days after inoculation on November 17, 1935. Of the 91 inoculated trees 86, or 95.5 per cent, showed infection. The inoculum was readily recovered in pure culture in a number of tests. The infection had progressed in nearly all instances from the point of inoculation on the small lateral root into the crown, and in a few cases the crown had been almost completely girdled. Many of these crowns ranged between 2 and 2½ inches in diameter. The rapidity and extent of the invasion and the high incidence of infection were particularly noteworthy. They afforded ample proof of the vigorous pathogenicity of the fungus under the conditions of the experiment. As noted previously the trees had been established under good conditions for growth 2 years prior to inoculation.

The response of the different rootstocks is presented in detail in table 2. The low incidence of infection resulting from the first series of inoculations warrants no conclusions as to varietal susceptibility; but the second series indicates very clearly that no one of them exhibits any measure of resistance to the strain of the fungus employed.

SUMMARY

High incidence of black root rot occurred within 2 and 3 years on young apple trees planted in thoroughly infested orchard soils. Of 165 trees under exposure, 100 (60.6 per cent) developed infection ranging from single lesions to as many as 10 separate lesions, and extensive invasion of the roots and crowns.

No evidence of any promising measure of resistance was exhibited by any one of 45 clons of *Malus* spp. exposed to natural infection, or by any one of 12 clonal stocks and 11 seedling stocks inoculated with *Xylaria mali* in pure culture. In one series of inoculations, 86 of 91 trees (95.5 per cent) became infected.

CONTACT PERIODS IN GRAFT TRANSMISSION OF PEACH VIRUSES

L. O. KUNKEL

(Accepted for publication April 5, 1938)

INTRODUCTION

None of the peach-virus diseases of the United States have been transmitted mechanically except by some form of grafting. Yellows, little-peach, rosette, and mosaic viruses are readily passed by either buds or scions. It is generally recognized that, in order to get transmission, grafted tissues must unite with the tissues of the stock. Buds and scions that die usually fail to infect. However, when they do not succumb promptly following the budding and grafting operations, they occasionally transmit disease. Insofar as the writer is aware, the period of contact required for transmission by means of buds or scions has not been reported for any plant virus. The question as to how long transplanted peach tissues must remain in contact with healthy tissues in order to obtain transfer of virus arose in connection with studies on the 4 diseases mentioned above. The further question as to whether or not each of the viruses would require the same period of contact was also considered. Experiments made for the purpose of attacking these problems have been completed and are reported below.

METHODS

Two methods were employed in attempts to determine the contact period necessary for transmission of peach yellows. In a few preliminary experiments both buds and scions were used in making inoculations. Since buds were easier to transplant than scions, and since nearly all buds lived, while an occasional scion died from mechanical injuries received when trees were watered, the former were employed in subsequent experiments.

The first method to be given a trial was the following: A diseased bud was inserted in the stem of each of several trees at a point about 6 inches above the soil level. After varying periods of time, the stems were cut off just below the inserted buds. As it was desirable to cut the stems close to the buds, but important that none of the infected bud tissues should be left on the stumps, each cut stem was examined to determine whether or not the nose of the bud had been clipped accidentally. In case the bud had been severed, a small portion of the stump was cut off. The trees were usually cut about 1 mm. below the transplanted tissues. After some days sprouts grew from the stumps. If virus had been transmitted, the sprouts showed the symptoms of yellows; if not, they were normal in appearance. In order to obtain transmission, it was necessary for virus to pass into the tree and move down the stem to a point below that at which the cut was made. After using this method in several experiments, it was abandoned because stumps occasionally died without producing sprouts.

The method finally adopted was to insert diseased buds in the stems of healthy trees, leave them for different periods of time, and then remove them. No procedure was found by which it was possible to remove completely the implanted bud tissues without injury to adjacent stock tissues. Removal of buds was accomplished in the following manner. The blade of a knife was used to lift the tail of the bud sufficiently to permit its being grasped by the thumb and forefinger. The bud was then stripped from its position by a sudden jerk. The bark that had covered the bud was cut off and the portion of the stem that had been in contact with the bud was scraped with a knife. This was done in order to remove any bits of bud tissue that might have adhered either to the bark or the stem of the tree. These operations destroyed all of the tissues that had been in immediate contact with the bud. Any bud causing infection had to remain in place long enough to allow the passage of virus into tissues that were not removed by the knife.

All buds were wooded and those used in any particular experiment were taken from the same tree. Since healthy and diseased meristematic tissues were pressed tightly together over the area of the stem covered by the bud, it was expected that the contact period required for transmission would be the same, or approximately so, in different trees and in different experiments. When this expectation was not realized in tests by means of single-bud inoculations, 5 buds and later 10 buds were inserted in each tree. However, the multiple-bud method of inoculation did not materially improve uniformity of results and was soon discontinued.

Potted seedling peach trees of about the same size and in the same state of vigor were used in each experiment. The ages of the trees in different experiments varied from 6 months to 2 years, but those used in any given experiment were of the same age. Only trees that were in prime condition for budding were inoculated. The same procedure was used in determining the contact periods necessary for transmission of little-peach, rosette, and mosaic viruses. All of the viruses were from sources metioned in previous papers.[1] [2]

EXPERIMENTAL RESULTS

Experiments to determine the contact period necessary for transmission of yellows were begun in January, 1930. Trees that had started growth following a dormant period in cold frames were used. In the first experiment 5 lots of 10 trees each were inoculated with yellows buds. One tree in each lot was cut off immediately below the point of inoculation each day, for a period of 10 days following the budding operation. None of the trees cut off within 4 days after budding were infected. Two of the 5 trees cut 5 days after budding and 4 of the 5 cut 6 days after budding became diseased.

[1] Kunkel, L. O. Immunological studies on the three peach diseases, yellows, rosette, and little peach. Phytopath. 26: 201–219. 1936.

[2] ———. Peach mosaic not cured by heat treatments. Am. Jour. Bot. 23: 683–686. 1936.

All that were cut 7, 8, 9, and 10 days after budding took yellows. Trees from one of the sets are presented in figure 1. The experiment showed that yellows virus did not pass from diseased buds into tissues below the points at which the trees were cut in as short a period as 4 days, that it sometimes passed in 5 days and that it usually passed in 6 or 7 days.

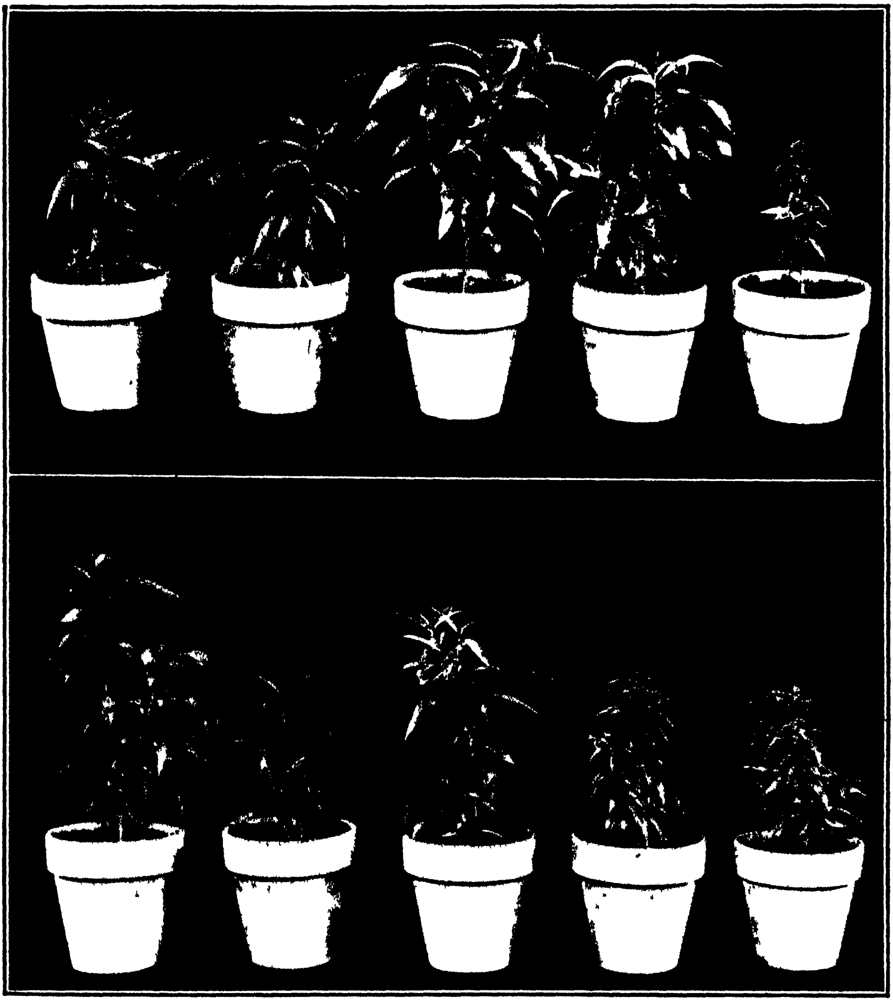

FIG. 1. Ten peach seedlings inoculated with yellows buds and cut off below points of insertion of buds after varying periods of time. Trees in the upper row were cut 1, 2, 3, 4, and 5 days after inoculation; trees in the lower row 6, 7, 8, 9, and 10 days after inoculation. The trees cut 1, 2, 3, 4, and 6 days after inoculation remained healthy; all others became diseased, although the second and third trees in the lower row did not show marked symptoms when the picture was taken. Virus passed into the last tree in the upper row after a contact period of 5 days.

In further experiments made later in 1930, longer contact periods were required. In several instances virus failed to pass during periods of 10 days. Seventeen different experiments, similar to the experiment described

above, but differing in that contact periods were terminated by removing the transplanted buds rather than by cutting the trees and differing in the number of trees used in some instances, have been made since 1930. The results obtained show that the contact periods necessary for transmission vary with the season of the year and with the age and condition of the trees inoculated. The shortest periods were obtained in early spring when trees were growing fastest. As the season advanced, the periods lengthened. In only one instance was yellows transmitted during a contact period as short as 3 days. The tree giving this minimum period was inoculated on February 18. In an experiment started February 20, transmission was obtained in 1 tree following a contact period of 4 days. On several occasions transmission was secured following contact periods of 5, 6, and 7 days, but longer intervals usually were required. Fairly uniform results were obtained with trees of the same age inoculated on the same date, but, even after the results of many experiments were known, it was not possible to predict accurately what the minimum period of contact would be for any given set of trees. However, the approximate length of the period was predictable. Under the favorable growing conditions of early spring, yellows virus always passed in from 3 to 7 days. At other seasons of the year it usually required from 8 to 14 days.

Results with little peach were similar to those with yellows. This virus usually required from 8 to 14 days to pass from little peach buds into healthy trees. The shortest period that permitted transmission was 5 days. After evidence was obtained that yellows and little-peach viruses are closely related,[3] experiments with the latter were discontinued.

The results with rosette closely paralleled those with yellows and little peach. In an experiment started February 19, transmission was secured after a contact period of 4 days. In 1 of 2 other experiments started on the same date, the minimum period was 5 days. Much longer contact periods usually were required.

The results obtained with yellows, little-peach, and rosette viruses showed that each needed about the same period of contact for transmission. They usually required about 5 days in early spring and about 10 days at other seasons of the year.

Peach-mosaic virus was first used in experiments carried out in 1936. In early spring it frequently was transmitted after contact periods of 2 days. This was shorter than the shortest contact periods that gave transmission of yellows, little peach, and rosette. At other seasons of the year, mosaic always was transmitted after much shorter intervals than the other viruses. However, it never passed in less than 2 days.

The difference in the behavior of mosaic virus and yellows and rosette viruses was most striking in tests carried out during seasons of the year when the latter required rather long periods of contact. The results of 3 experiments with the 3 viruses are presented in table 1. The first of the experi-

[3] See footnote 1.

TABLE 1.—*Contact periods required for transmission of yellows, rosette, and mosaic viruses*

Contact periods in days	Experiment 1			Experiment 2			Experiment 3		
	Trees budded with			Trees budded with			Trees budded with		
	Yellows	Rosette	Mosaic	Yellows	Rosette	Mosaic	Yellows	Rosette	Mosaic
1	-	-	-	- - - -	- - - -	- - - -	- - - - -	- - - - -	- - - - -
2	-	-	-	- - - -	- - - -	- * * -	- - - - -	- - - - -	- - - - -
3	-	-	*	- - - -	- - - -	* - * *	- - - - -	- - - - -	* - - * -
4	-	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * - * *
5	-	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * * * -
6	-	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * * * *
7	-	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * * * *
8	-	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * * * *
9	+	-	*	- - - -	- - - -	* * * *	- - - - -	- - - - -	* * * * *
10	-	-	*	- - × -	- - - -	* * * *	+ + + + +	× × × × ×	* * * * *
11	-	-	*	+ - + +	- - - -	* * * *	+ + + + +	× × × × ×	* * * * *
12	-	-	*	+ + + +	- - - ×	* * * *	+ + + + +	× × × × ×	* * * * *
13	+	×	*	+ + + +	× × - ×	* * * *	+ + + + +	× × × × ×	* * * * *
14	+	×	*	+ + + +	× × × ×	* * * *	+ + + + +	× × × × ×	* * * * *
15	+	×	*						
16	+	×	*						
17	+	×	*						
18	+	×	*						
19	+	×	*						
20	+	×	*						

+ = yellows infection.
× = rosette infection.
* = mosaic infection.
– = no infection.

ments was started on April 18, the second on June 22, and the third on September 7. In each of the experiments mosaic required much shorter periods of contact than either yellows or rosette. In the first, mosaic was transmitted after 3 days, while yellows and rosette were not regularly transmitted in less than 13 days. In the second experiment mosaic was transmitted to 2 trees with contact periods of 2 days, to 3 trees with contact periods of 3 days, and to all trees with contact periods of 4 days or longer, while yellows and rosette required from 10 to 14 days. In the third experiment mosaic was transmitted to 2 trees with contact periods of 3 days and to all trees with contact periods as long as 6 days, while both yellows and rosette required 10 days.

DISCUSSION

The experiments reported in this paper show that yellows, little-peach, and rosette viruses usually were transmitted only after rather long periods of contact between diseased and healthy tissues. Periods of about 10 days were required during all seasons of the year except early spring. The minimum periods obtained by early spring budding were 3 days for yellows, 5 days for little peach, and 4 days for rosette. The differences in the lengths of the minimum periods are believed not to be significant, since each of these viruses usually passed in about the same period of time.

While the minimum period of contact necessary for the transmission of mosaic was only 1 day shorter than the corresponding minimum period found for yellows, the difference is believed to be significant. Under the same or similar conditions, mosaic was always transmitted after shorter periods, and usually after much shorter periods, than were the other viruses.

The suggestion frequently has been made that viruses may pass from cell to cell through plasmodesmata.[4] It was thought that the period of contact required for transmission of a virus might represent the time necessary for the establishment of plasmodesmata between diseased and healthy cells. If this were true, it would seem that, under similar conditions, the 4 peach viruses should require about the same periods of contact. The fact that yellows, little-peach, and rosette viruses usually required 4 or 5 times as long as mosaic virus to pass from diseased buds into healthy stems suggests that plasmodesmatal connections may be made more quickly between healthy and mosaic-affected tissues than between healthy and yellows-affected tissues, healthy and little-peach-affected tissues, or healthy and rosette-affected tissues, but this may not be the correct explanation. Some property inherent in the mosaic virus may account for the short contact period required for its transmission.

SUMMARY

Contact periods required for transmission of the viruses causing yellows, little peach, rosette, and mosaic of peach by budding are reported. Mosaic

[4] Livingston, L. G. The nature and distribution of plasmodesmata in the tobacco plant. Am. Jour. Bot. 22: 75–87. 1935.

virus usually passed in from 2 to 3 days, the other viruses in from 8 to 14 days. If transfer of mosaic virus depends on the development of plasmodesmatal connections between diseased and healthy tissues, these connections must sometimes be established between mosaic and healthy tissues within a period of 2 days.

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DEVELOPMENT OF A DIFFERENTIAL INOCULATION TECHNIQUE FOR DIPLODIA STALK ROT OF CORN[1]

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(Accepted for publication March 8, 1938)

Among the most important disease problems of dent corn, *Zea mays* L., is a complex of stalk disorders commonly referred to as stalk rot. As implied in the preceding sentence, the term stalk rot is an inclusive one from the standpoint of causal relationships. From the standpoint of symptoms or ultimate effects the term has, however, a rather definite meaning to plant breeders generally, and is used in reference to stalk breaking when it occurs below the ear. The resultant lodging is distinguishable from that caused by poor root anchorage or root diseases.

The modern method of corn improvement, which involves hybridization among inbred lines, offers the most practical means for the control of the stalk rots through breeding for resistance to these diseases. That considerable progress has been made in the control of stalk rots is evidenced by the minimum of stalk breaking observed in many hybrids now in commercial production. In the development of their inbred lines, breeders have discriminated severely against stalk breaking, with the result that the relatively high level of resistance to stalk rot attained in the recombinations is a common and distinguishing feature of hybrid corn generally. This is a characteristic to which must be credited in no small measure the reason for the general popularity of hybrid corn.

A rapid method of determining relative resistance to stalk rot would be very useful to the corn breeder, and would in all probability stimulate more direct attention to this important disease problem in the breeding practices. A rapid method of measuring relative resistance also would facilitate needed studies on the nature of resistance and on other fundamental aspects.

[1] Received for publication Feb. 25, 1938; issued July 15, 1938. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station and Funk Bros. Seed Co.

[2] The writers are indebted to Mr. Eugene H. Herrling, of the Department of Plant Pathology, University of Wisconsin, for the drawing in figure 1.

The parasitic relationships involved in the stalk-rot complex have not been clearly defined. Of the various fungi believed to be directly associated with stalk rots, *Diplodia zeae* (Schw.) Lév. probably is of greatest importance under Illinois conditions. The rôle of *D. zeae* as a primary parasite causing stalk rot and stalk breaking has been established from adequate inoculation experiments.

The data presented herein were obtained in an artificial inoculation experiment conducted near Bloomington, Illinois, in 1931, and concern initial studies on the development of a differential inoculation technique for the Diplodia stalk rot of corn.

MATERIALS AND METHODS

Thirteen single crosses representing various combinations among 9 different inbred lines of dent corn were used. The hybrids were planted across a strip of new-plowed virgin prairie soil in drilled rows about 60 feet long. The individual plants were spaced about 1 foot apart in the rows. The first replication consisted of 2-row plots, while additional replications of the hybrids were planted in single rows. The plants in the first row of the 2-row plots were artificially inoculated and those in the second row were reserved for notes on stalk rot resulting from natural causes or natural sources of infection. The additional single-row replications were needed to provide the larger populations of noninoculated plants believed necessary to obtaining data on stalk rot from natural sources and also for additional inoculations in certain of the hybrids, as will be explained later.

Resistance to stalk rot is relative, and varying degrees of resistance or of susceptibility usually may be found within any arbitrary grouping of lines. This holds in the following classification for the 9 inbreds involved in the hybrids studied in this experiment: Resistant, *Hy;* susceptible, *Lan, R313,* and *H;* intermediate, *B, 90, A18, A,* and *L.* The classification of these inbreds is based on the reactions of the lines themselves to stalk rot from natural causes as observed in former years.

Inoculations were made during the last week in August, or about 3 or 4 weeks after the pollination period, in the following manner. A puncture was made through the center of a lower internode, usually about 1 foot above the soil line, with a steel needle about 2 millimeters in diameter. Inoculum consisting of a water suspension of pycnospores[3] from a monospore culture of *Diplodia zeae* was then injected into the puncture by means of a syringe having a glass-tube outlet.

Final notes were taken over a period of several days during the second week in October. Data taken on the inoculated plants involved 2 different measurements of disease, (1) pith spread, and (2) cortical spread.

Pith Spread. Determined after splitting the stalks longitudinally with a knife and then measuring the linear distance of spread of the rot from the

[3] Cultures grown on oatmeal agar incubated for about 6 weeks at room temperature produced abundant pycnidia.

point of inoculation. The pith readings were calculated in terms of the percentage of the distance between the base of the plant and the first ear.

Cortical Spread. A measure of the surface area of the outer cortex visibly rotted or discolored. The cortical readings also were expressed in terms of the percentage of the surface area between the base of the plant and the first ear.

Data taken on the noninoculated plants included (1) percentage of plants whose stalks showed evidence of natural infection with stalk rot; and (2) percentage of plants with stalks broken beneath the point of ear attachment. Thus, 4 different measurements of amount of disease, or of relative resistance, were obtained for each of the 13 hybrids; pith spread and cortical spread for the inoculated plants, and natural infection and broken stalks for the noninoculated controls. The correlations among the results from these different methods of measuring disease are used as the criterion for judging the efficiency of the artificial inoculation method as a means for determining relative resistance.

All badly smutted, barren, and otherwise undesirable plants in both the inoculated and the noninoculated series were discarded from consideration when final notes were taken. All plants in the artificially inoculated rows whose stalks showed evidence of natural infection with *Diplodia zeae* or other stalk rot fungi, likewise were discarded. Since the mortality from this source was expected to be high, especially in susceptible hybrids, an additional number of inoculations were made in some of the replicated plots to insure sufficiently large populations.

TABLE 1.—*Reactions of 13 dent corn single crosses to stalk rot from natural sources of infection, and to stalk rot following artificial inoculation with Diplodia zeae, at Bloomington, Ill., 1931*

Hybrid	Noninoculated				Inoculated		
	Natural infection		Broken stalks				
	Plants examined	Plants infected	Plants examined	Plants broken	Number of plants	Pith spread[a]	Cortical spread[b]
	No.	*Per cent*	*No.*	*Per cent*			
A x Hy	32	3.1	85	7.1	32	14.1	1.9
Hy x L	30	6.6	55	7.3	46	23.5	3.2
H x Hy	25	4.0	123	5.6	30	17.4	4.4
B x Hy	6	16.7	8	0.0	19	39.9	8.6
B x 90	45	15.5	65	7.6	45	37.6	11.9
A x L	31	45.1	90	28.9	50	33.6	12.0
A48 x L	29	3.4	117	10.2	44	28.1	12.3
B x H	31	32.2	46	39.1	23	32.3	15.7
90 x R313	26	23.1	128	44.5	45	41.6	27.8
Lan x 90	30	30.0	83	28.9	45	58.9	39.5
H x 90	31	51.6	80	50.0	50	53.9	43.7
Lan x H	33	69.7	61	59.0	46	58.0	45.5
Lan x R313	32	81.2	88	69.3	47	74.6	68.6

[a] Linear measurement of pith tissues rotted are in terms of the percentage of the distance between the base of the plant and the first ear.

[b] Surface area of the cortex rotted as observed from external appearances are in terms of the percentage of the total surface area between the base of the plant and the first ear.

RESULTS

The data for both the inoculated and the noninoculated populations of the 13 single crosses studied are summarized in table 1.

Correlations Between Results from Inoculated and Noninoculated Populations

The data in table 1 show that wide differences in reaction obtained among the hybrids in both the inoculated and the noninoculated series. In order that the comparison of the results between the inoculated and the noninoculated series may be facilitated, the data for each hybrid are reassembled and

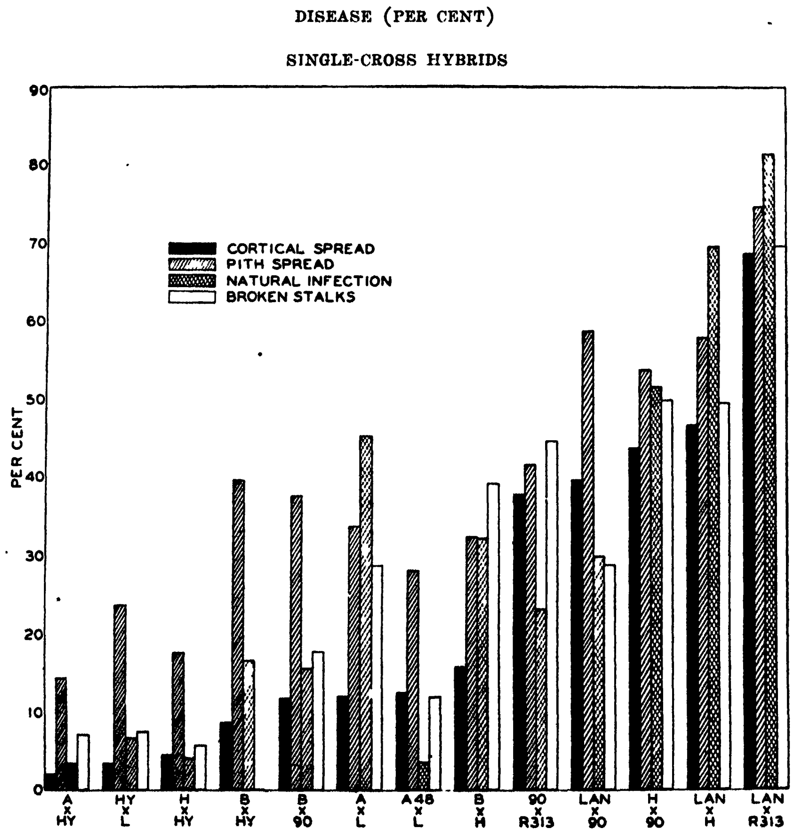

FIG. 1. Comparative amounts of stalk rot in noninoculated plants and in plants artificially inoculated with *Diplodia zeae* in each of 13 single crosses of dent corn. Cortical spread and pith spread are measurements of stalk rot for the inoculated plants; natural infection and broken stalks are measurements for the noninoculated plants. (Data in table 1.)

shown graphically in figure 1. The correlation coefficients are given in table 2.

The correlation between pith spread and cortical spread (inoculated plants) is + 0.948 and between natural infection and broken stalks (noninoculated plants) is + 0.909. These high, positive correlations indicate

TABLE 2.—*Correlation coefficients between various measurements of amount of stalk rot in inoculated and in noninoculated populations of 13 single-cross dent corn hybrids*

Character	Pith spread	Cortical spread	Broken stalks
Cortical spread	+ 0.948		
Broken stalks	+ 0.821	+ 0.899	
Natural infection	+ 0.853	+ 0.878	+ 0.909

there was little difference between the measurements of disease for the respective populations. Comparing the results from the inoculated series with those from the noninoculated series it is seen in table 2 that the correlations between pith spread and broken stalks, and between pith spread and natural infection are + 0.821 and + 0.853, respectively. The correlation between cortical spread and broken stalks is + 0.899; and between cortical spread and natural infection is + 0.878. It is apparent from the magnitude of these correlations that the results from the artificially inoculated series approximated very closely those obtained from the noninoculated controls. Stated differently, the relative resistance of the hybrids studied in this experiment was measured to a satisfactory degree of accuracy by means of artificial inoculation.

Influence of Inbreds upon Hybrid Reaction

The positive influence of inbreds upon hybrid reaction is illustrated in the results obtained (Fig. 1). The 4 different hybrids having the resistant

FIG. 2. Hybrid H × 90 following inoculation of the stalks with *Diplodia zeae*.

inbred *Hy* as one of the parents ranked highest in resistance for cortical spread. In contrast, the 2 hybrids that combined 2 susceptible inbreds ranked lowest in resistance. The 1 hybrid involving a highly resistant and a very susceptible inbred ($H \times Hy$) reacted resistant, indicating a dominance of resistance in this cross. Field photographs illustrating typical reactions of resistant and of susceptible hybrids, to artificial inoculation and to stalk rot from natural sources, are shown in figures 2 to 4.

FIG. 3. The same hybrid as in figure 2 (row in left foreground) noninoculated. Note the evidence of this hybrid's susceptibility to stalk rot as indicated by its reaction to artificial inoculation, and here by the large number of broken stalks which resulted from natural causes or natural sources of infection. Compare with photograph in figure 4.

DISCUSSION

The most promising aspect in the results from the inoculation studies described is the evidence that reaction to Diplodia stalk rot can be measured by means of artificial inoculation. The results obtained also indicate that reaction to stalk rot is hereditary in nature. These facts are of basic importance to the problem of the development of a useful differential inoculation technique for breeders.

A promising lead for studies on the nature of resistance is suggested by the differences shown in the ability of the hybrids to restrict the advance of the fungus in their stalk tissues. Preliminary histological studies by the senior writer[4] showed rather striking differences between a hybrid having

[4] Smith, A. L. The reactions of inbred and hybrid strains of yellow dent corn to infection by *Diplodia zeae* (Schw.) Lév. (Unpublished thesis) 1932.

FIG. 4. Hybrid *H* × *Hy* following inoculation of the stalks with *Diplodia zeae* (left) and its reaction to stalk rot from natural sources of infection (right). The influence of the resistant inbred *Hy* is shown in the comparison of the photographs in figures 2 and 3.

the resistant inbred *Hy* as one of its parents, and one having the susceptible inbred *Lan* as a parent, with respect to the relative amounts of sclerenchyma tissues and the thickness of cell walls in parenchymatous tissues. The studies are considered too limited for any general conclusions but, nevertheless, are very suggestive.

The data presented herein on the reactions of the single-cross hybrids represent results from but a single experiment and are taken from the unpublished thesis referred to above. It should be stated, however, that in the course of the thesis studies in experiments in which the matter of method of inoculation was stressed particularly, a very considerable amount of observational evidence was obtained that resistance to Diplodia stalk rot can be measured by means of artificial inoculation. Additional and quite conclusive evidence has since been obtained by the junior writers in subsequent studies involving the application of the artificial method of inoculation to special problems.

It was realized at the time these studies were completed that the accomplishment of the differential inoculation technique marked a good beginning, but that many studies yet remained to be made before the method could be recommended for practical application. At the present time the inocu-

lation problem has resolved itself into a number of specialized studies. Among things to be considered, the relationship between Diploidia stalk rot and other stalk rots is of utmost importance. How extensively does Diploidia stalk rot occur? Would resistance to Diplodia stalk rot mean resistance to other stalk rots; and, if this should prove to be true, will it be possible to use *Diplodia* in artificial inoculations as a general tester for stalk rot resistance? Another very important problem concerns possible relationships between susceptibility to stalk rot and factors for yield. The questions raised must be answered with definite information in order to know just when and where the inoculation method may be used to advantage. There also remain problems in technique, such as those pertaining to size of population, stage of maturity for inoculating, and others that likewise must be investigated before the artificial method for evaluating resistance may be considered as a usable tool in the breeding program.

SUMMARY

Data are presented from studies on the development of an inoculation technique for the Diplodia stalk rot of corn to which the strains tested showed a marked differential in the amount of stalk rot that developed. The methods used in making inoculations and in taking data are described in detail.

The amount or extent of stalk rot following artificial inoculation is compared with the extent of stalk rot resulting from natural sources of infection in each of 13 dent corn single crosses. The correlations obtained in the comparisons are used as a basis for judging the efficiency of the artificial method of inoculation in measuring relative resistance.

The measurements of disease in the inoculated plants are (1) pith spread, and (2) cortical spread; and in the noninoculated plants are (1) natural infection, and (2) broken stalks. The correlation coefficients obtained between these various measurements for disease are as follows: pith spread and cortical spread, + 0.948; natural infection and broken stalks, + 0.909, pith spread and natural infection, + 0.853; pith spread and broken stalks, + 0.821; cortical spread and natural infection, + 0.878; cortical spread and broken stalks, + 0.899.

Data indicating the genetic or hereditary nature of reaction to stalk rot are presented.

It is concluded that relative resistance to Diplodia stalk rot can be measured by means of artificial inoculation with the fungus. Problems are mentioned, which need to be investigated, however, before the inoculation technique can be used as a tool in agronomic practice.

VARIETAL REACTION OF PEA TO A VIRUS FROM ALSIKE CLOVER

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(Accepted for publication March 14, 193?)

INTRODUCTION

In 1936 the writers reported a virus from alsike clover, *Trifolium hybridum* L., that was infectious to pea, *Pisum sativum* L. This virus was collected in the mountainous regions of northeastern Colorado, where it was commonly found affecting alsike clover.

This virus was shown by Zaumeyer and Wade[1] to be unlike those previously reported to be infectious to pea. Certain varietal studies were made earlier, but difficulty was experienced in securing sufficiently consistent percentages of infection for a significant determination of relative susceptibility or resistance. Since then, the use of carborundum powder as an abrasive in inoculating has made it possible to secure more consistent and dependable degrees of infection.

The purpose of this paper is to record the reaction of a number of pea varieties to this virus.

SYMPTOMS

The symptoms produced by this disease have been described earlier.[1] In general they resemble those produced by the common pea mosaic virus (*pea virus 3*).[2] With the exception of a few varieties such as Dwarf Telephone, Potlatch, Alderman and similar ones, no appreciable stunting of the plants is noted. A distinct mottling is characteristic of varieties such as Alaska, Dwarf Grey Sugar, Green Admiral, and Austrian Winter, while pronounced chlorosis is exhibited on the varieties Gradus, Stratagem, Prince of Wales, and Giant Stride.

METHODS

These studies were conducted under field conditions in Colorado, both in 1935 and 1936. Replicate plots of 111 varieties or strains of peas were grown each year. Fifty seeds of each variety (Table 1) were planted in each of 2 series, and the Broad Windsor bean, *Vicia faba* L., was systematically planted throughout the plot. This host is extremely susceptible to the virus and an excellent plant on which to rear the pea aphid, *Macrosiphum pisi* Kalt., which transmits the disease.

The virus inoculum was prepared by passing mosaic-infected alsike clover plants through a meat grinder. Carborundum powder was added to the expressed juice, which was slightly diluted with water. The inoculum so prepared was rubbed on the leaves of the plants by means of cheesecloth

[1] Zaumeyer, W. J. and B. L. Wade. Pea mosaic and its relation to other legume viruses. Jour. Agr. Res. [U. S.] 53: 161–185. 1936.

[2] Pierce, W. H. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. 51: (1935) [U. S.] 1017–1039. 1936.

TABLE 1.—*The reaction of varieties of peas to alsike virus 1*

Variety	Survival values:		
	1935	*1936*	*Avg.*
American Wonder (2)[a]	4.00	4.00	4.00
Ashford	4.00	4.00	4.00
Blue Bantam	4.00	4.00	4.00
Canner Gem (2)[a]	4.00	4.00	4.00
Delikatess	4.00	4.00	4.00
Dwarf Gray Sugar[b]	4.00	4.00	4.00
Excelsior, Nott	4.00	4.00	4.00
Excelsior, Sutton	4.00	4.00	4.00
Horal	4.00	4.00	4.00
Little Marvel	4.00	4.00	4.00
Perfection (2)[a]	4.00	4.00	4.00
Perfection, Renard	4.00	4.00	4.00
Perfection, Root Rot Resistant	4.00	4.00	4.00
Perfection, Wilt Resistant	4.00	4.00	4.00
Perfection, Wisconsin	4.00	4.00	4.00
Premium Gem (2)[a]	4.00	4.00	4.00
Short Admiral	4.00	4.00	4.00
Superb	4.00	4.00	4.00
Surprise	4.00	4.00	4.00
Wisconsin Early Sweet	4.00	4.00	4.00
Morse Market	3.95	4.00	3.98
White Eye Marrowfat[b]	3.85	3.85	3.85
Black Eye Marrowfat[b]	4.00	3.65	3.83
Horsford[b]	3.60	4.00	3.80
Hundredfold[b]	3.90	3.50	3.70
Progress	3.45	3.50	3.48
Laxton Superb	4.00	1.05	2.53
Chancelot	4.00	1.00	2.50
Hundredfold[b]	4.00	1.00	2.50
Dwarf Alderman[b]	2.85	1.80	2.33
White Eye Marrowfat[b]	3.65	1.00	2.33
Carter Eight Weeks	3.15	1.15	2.15
Champion of England	3.30	1.00	2.15
Maple[b]	3.10	1.15	2.13
Long Pod Alaska	3.10	1.10	2.10
Meteor	3.05	1.10	2.08
Progress	2.65	1.45	2.05
Lock Goil	3.05	1.00	2.03
Stella	3.00	1.05	2.03
Blue Bell	3.00	1.00	2.00
Bruce	2.80	1.15	1.98
Morse #200	2.95	1.00	1.98
Alaska	2.70	1.15	1.93
Lincoln	2.70	1.10	1.90
Phenomenon	2.15	1.65	1.90
Yellow Admiral[b]	2.00	1.75	1.88
Dwarf Alderman[b]	2.65	1.05	1.85
Early Eight Weeks[b]	2.60	1.05	1.83
Banqueter	2.70	1.05	1.88
Winner[b]	2.65	1.00	1.83
Thomas Laxton[b]	2.55	1.05	1.80
Maple[b]	2.50	1.05	1.78
Perfectah	2.45	1.05	1.75
Md. Alaska	2.35	1.05	1.70
Harrison Glory	2.25	1.05	1.65
Maple[b]	2.25	1.05	1.65
President Wilson	1.90	1.35	1.63
Sugar	2.20	1.05	1.63

[a] Numbers in parenthesis represent number of apparently identical strains

[b] Other strains of the same variety gave different results.

TABLE 1.—(*Continued*)

Variety	Survival values:		
	1935	*1936*	*Avg.*
Duplex	2.05	1.15	1.60
Mammoth Melting Sugar	2.10	1.10	1.60
Senator[b]	2.00	1.15	1.58
Green Admiral[b]	2.00	1.10	1.55
Horsford[b]	1.95	1.15	1.55
Pride of the Market	1.95	1.15	1.55
Winner[b]	2.00	1.10	1.55
Bountiful	1.65	1.40	1.53
White Canada	2.00	1.00	1.50
Green Admiral[b]	1.80	1.10	1.45
Yellow Admiral[b]	1.85	1.05	1.45
Improved Hundredfold	1.85	1.00	1.43
Gilbo	1.80	1.00	1.40
Bluebell	1.65	1.10	1.38
Stratagem[b]	1.65	1.10	1.38
Green Giant[b]	1.70	1.00	1.35
Dwarf Gray Sugar[b]	1.50	1.15	1.33
Profusion	1.55	1.10	1.33
Alderman[b]	1.55	1.05	1.30
World Record[b]	1.50	1.10	1.30
Alderman[b]	1.55	1.00	1.28
Prince of Wales[b]	1.55	1.00	1.28
Rice No. 13	1.50	1.00	1.25
Swedish Yellow	1.50	1.00	1.25
V C	1.50	1.00	1.25
Abundance	1.45	1.00	1.23
Pioneer[b]	1.35	1.10	1.23
Potlatch	1.45	1.00	1.23
Stratagem[b]	1.40	1.00	1.20
Frost Resistant Stratagem	1.25	1.15	1.20
Ameer	1.25	1.05	1.15
Gradus[b]	1.00	1.30	1.15
Improved Pilot	1.25	1.00	1.13
Kootenay	1.05	1.15	1.10
Dairy[b]	1.00	1.10	1.05
Austrian Winter[b]	1.00	1.05	1.03
British Lion	1.00	1.05	1.03
Creole	1.00	1.05	1.03
Giant Stride	1.05	1.00	1.03
Gradus[b]	1.00	1.05	1.03
Tall Telephone	1.00	1.05	1.03
Daisy	1.00	1.00	1.00
Duke of Albany	1.00	1.00	1.00
Dwarf Quite Content	1.00	1.00	1.00
Edible Pod	1.00	1.00	1.00
Everbearing	1.00	1.00	1.00
G. O. P.	1.00	1.00	1.00
Late Gradus	1.00	1.00	1.00
Late Duke	4.00		
Onward	4.00		
Premium Gem	4.00		
Charles the First	3.95		
Carter Late Giant	3.75		
Extra Early	3.25		
Klevendon Wonder		4.00	
Lord Chancellor		4.00	
Black Eye Marrowfat[b]	2.90		
World Record[b]	2.25		

[a] Numbers in parenthesis represent number of apparently identical strains.
[b] Other strains of the same variety gave different results.

TABLE 1.—(*Continued*)

Variety	Survival values: 1935	1936	Avg.
Alderman[b]	2.20		
Thomas Laxton[b]	2.10		
World Record[b]	2.05		
Capucijner	2.00		
Mammoth Dwarf Sugar	2.00		
Maple[b]	2.00		
Prince of Wales[b]	2.00		
Yorkshire Hero	1.95		
Marrowfat	1.80		
Half Dwarf Melting Sugar	1.65		
Quite Content	1.65		
World Record[b]	1.65		
Senator[b]	1.55		
Austrian Winter[b]	1.25		
Mammoth Melting Sugar	1.25		
Admiral Beatty	1.00		
Moerheim Giant		1.10	
Early Eight Weeks[b]		1.05	
Green Giant[b]		1.05	
Japanese Pea		1.05	
Pioneer[b]		1.05	
Sutton Foremost		1.05	
Late Alaska		1.00	
Zwann's Paramount		1.00	

[a] Numbers in parenthesis represent number of apparently identical strains.
[b] Other strains of the same variety gave different results.

pads. Alternate plants in each lot were inoculated when about 6 inches high. The pea aphids that migrated to the peas and the Broad Windsor bean and multiplied there were not controlled. They never reached such numbers that direct aphid injury to the peas resulted, but they were responsible for much of the secondary dissemination of the disease.

A plant showing no symptoms at the end of about 10 weeks was recorded as healthy or resistant and given a rating of 4; mildly infected plants, 3; more severely infected, 2; and very severely, 1. No killing of the plants by the disease occurred. The products of the numbers of plants in each category, multiplied by the survival value assigned that class, were summed to develop a total survival value of each plot. Since the number of plants varied from plot to plot, depending upon the germination, occasional death from root rot caused by *Fusarium martii* var. *pisi*, and infrequent damping off due to *Pythium* sp., the total survival value of each plot was divided by the number of plants in the plot and this average was recorded as the survival value of the plot. Table 1 shows the values for the averages of 2 plots in 1935, 2 in 1936, and the 2-year average for those varieties observed in both years.

EXPERIMENTAL RESULTS

In Table 1, the varieties are arranged in order of their indicated resistance to *alsike virus* 1, based on the average of the 4 plots in 2 years. Where ties

occur the arrangement is alphabetical. The 34 varieties grown only one year are included in table 1, but they are not included in the ranking with varieties grown 2 years.

The varieties of peas tested were randomized within blocks and the statistical constants computed in a variance-analysis arrangement (Table 2).

TABLE 2.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for 1935 and 1936*

Variation due to:	Degrees of freedom	Mean square
Varieties	110	5.25[a]
Years	1	46.77
Blocks	1	.08
Blocks × varieties	110	.18
Years × varieties	110	.64
Error	111	.16
Total	443	1.65

[a] F = 32.81 when referred to error. 1 per cent point = 1.39.
S.D = 0.40.
SE_{m_4} = 0.20. Significant difference = 0.57.

The standard error of the mean of the reaction of 4 plots was found to be 0.20. For differences in reaction to be considered significant, they should exceed 0.57. In addition to the 111 pea varieties (Table 1) grown in 1935 and 1936, 24 others were included in 1935 and 10 in 1936. Variance analyses for 135 and 121 varieties, respectively, are shown in tables 3 and 4. The standard error of the mean of 2 plots is 0.40 for 1935 and 0.13 for 1936, with

TABLE 3.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for 1935*

Variation due to:	Degrees of freedom	Mean square
Varieties	134	2.59[a]
Blocks	1	0.15
Error	174	.33
Total	309	1.31

[a] F = 7.82 when referred to error. 1 per cent point = 1.27.
S.D. = 0.57.
SE_{m_2} = .40. Significant difference = 1.13.

TABLE 4.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for for 1936*

Variation due to:	Degrees of freedom	Mean square
Varieties	120	3.32[a]
Blocks	1	0.10
Error	120	0.0314
Total	241	1.67

[a] F = 105.73 when referred to error. 1 per cent point = 1.32.
S.D. = 0.177.
SE_{m_2} = Significant difference = 0.35.

significant differences of 1.13 and 0.35, respectively. In 1935 Potlatch was planted 15 times in each series and Alderman 7 times. The standard error of the mean of 30 plots of Potlatch is 0.10 and of 14 plots of Alderman, 0.15.

Twenty-four varieties of peas showed complete freedom from the disease in both years (Table 1). It is of interest to note that many of these are Gem-type peas, or rather closely related to this type. All strains of Perfection, including the wilt (*Fusarium orthocerus* var. *pisi*)-resistant and wilt-susceptible types, proved to be completely resistant to the virus except Perfectah, which showed a 2-year survival value of 1.75. Abundance, closely related to Perfection, had a survival value of 1.45 for 2 years. Most of the mosaic-free varieties in the group are canning types.

All of these varieties are market-garden types. Seven varieties were completely susceptible in both years, while others showed high resistance in 1935 and high susceptibility in 1936. Laxton Superb, Chancelot, one strain of Hundredfold, White Eye Marrowfat, and others gave decidedly more varied reactions in 1935 than in 1936.

The behavior of Laxton Superb is attributed to difficulties in interpretation of symptoms. Healthy plants are frequently light colored and slightly mottled, thus resembling those that are infected with mosaic. The Hundredfold strains proved to be of 2 different types, susceptible and resistant, with admixtures of types within a given strain. One strain of White Eye Marrowfat was found to be a mixture of resistant and susceptible biotypes. Chancelot was not available for further study, which made it impossible to determine the reason for variation in results.

The influence of environmental conditions upon the incidence and expression of the disease is shown by the much higher level of survival in 1935 than in 1936. The survival value of the 111 strains was only 74.47 per cent as great in 1936 as in 1935. The mean survival value for 222 plots grown in 1935 was 2.54, but in 1936 it was 1.89 for the same varieties. The difference amounts to the highly significant difference of 0.65 ± 0.038. In general, however, those varieties that ranked high in 1935 also rated fairly high in 1936.

DISCUSSION

As mentioned previously, the symptoms produced by *alsike clover virus 1* on peas are quite similar to those caused by the common pea-mosaic virus (*pea virus 3*). It would be difficult if not impossible to differentiate these 2 diseases on symptomological characters alone. Certain varieties of peas, however, react differently when inoculated with the 2 viruses. In this manner, together with other characteristic features that will be described in a later paper, the 2 viruses can be differentiated.

Although 50 per cent of the plants were inoculated artificially with *alsike virus 1*, the pea aphid, likewise, transmitted the virus to a considerable percentage of the remaining plants. Because of this, it is probable that a small percentage of the plants may have been infected with other viruses. It is believed that the percentage of plants thus infected was small, since, had

other viruses been present, it is probable that they would have infected a small percentage of the 20 varieties that showed complete resistance in both years (Table 1). Furthermore, the highest percentage of aphids transmitting the virus would, most likely, be infected with *alsike virus 1*, because of the abundant amount of inoculum present in the pea field, and not with other viruses that may have been present on other related hosts growing in proximity to the peas. Aphids from such plants would have been the main source of this infection, which is believed to have been negligible.

Most varieties that show resistance to *alsike virus 1* are likewise resistant to the common pea-mosaic virus. Blue Bantam, Surprise, and some strains of Dwarf Gray Sugar are resistant to *alsike virus 1*, but susceptible to the common pea virus.[3] Conversely, Morse's Market, Rice's No. 13, White Eye Marrowfat, and Hundredfold peas resist the common pea virus,[3] but are susceptible to *alsike virus 1*, with the exception of some strains of Hundredfold.

Alsike virus 1 is differentiated from the pea enation virus (*pea virus 1*),[5] the white-clover-mosaic virus,[6] and the virus from white sweet clover (*bean virus 2*)[7] by differences in varietal reaction of certain pea varieties. Nott Excelsior, Horal, Perfection, Surprise, Wisconsin Early Sweet and Hundredfold varieties resist *alsike virus 1*, but are susceptible to *pea virus 1*.[8] These varieties, together with Little Marvel, Premium Gem, and Onward, also resist *alsike virus 1*, but are susceptible to the white clover virus and the sweet clover virus, with the exception of Horal and Perfection, which are resistant to the latter. The virus recently described by Osborn[9] as the vein mosaic virus of red clover, differs from *alsike virus 1* in that Horal and Perfection are susceptible to this virus.

SUMMARY

Field studies were conducted in 1935 and 1936 to determine the varietal susceptibility and resistance of 145 varieties and strains of pea to *alsike virus 1*. The statistical constants were computed in a variance analysis. Twenty-four strains showed complete freedom of the disease in both years, while 7 were completely susceptible. Many of the resistant strains are of the Gem type or rather closely related to this type. Although the survival value in 1935 was of a higher level than in 1936, in general those varieties that ranked high in 1935 also rated fairly high in 1936.

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UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

[3] Murphy, D. M. and W. H. Pierce. Common mosaic of the garden pea, *Pisum sativum*. Phytopath. 27: 710–721. 1937.
[4] See footnote 3.
[5] See footnote 2.
[6] See footnote 1.
[7] Pierce, W. H. Viroses of the bean. Phytopath. 24: 87–115. 1934.
[8] See footnote 2.
[9] Osborn, H. T. Vein mosaic virus of red clover. Phytopath. 27: 1051–1058. 1937.

ROT OF MATURE TAP ROOT OF SUGAR BEET CAUSED BY PYTHIUM BUTLERI[1]

W. A. KREUTZER AND L. W. DURRELL[2]

(Accepted for publication March 5, 1938)

In the late summer of 1936 the senior writer had occasion to observe a peculiar root malady of sugar beets, *Beta vulgaris* L., in a large field in the Rocky Ford district. The disease was characterized by a wilting, yellowing,

FIG. 1. A. Naturally infected field beet root showing the characteristic rot induced by *Pythium butleri*. B. Beet roots artificially inoculated with *P. butleri*. Note the mottled effect as shown by longitudinally cut roots.

[1] Published with the approval of the Director of the Colorado Agricultural Experiment Station.

[2] The writers wish to express their appreciation to Dr. Charles Drechsler for his identification and to Dr. W. J. Zaumeyer for his advice and assistance.

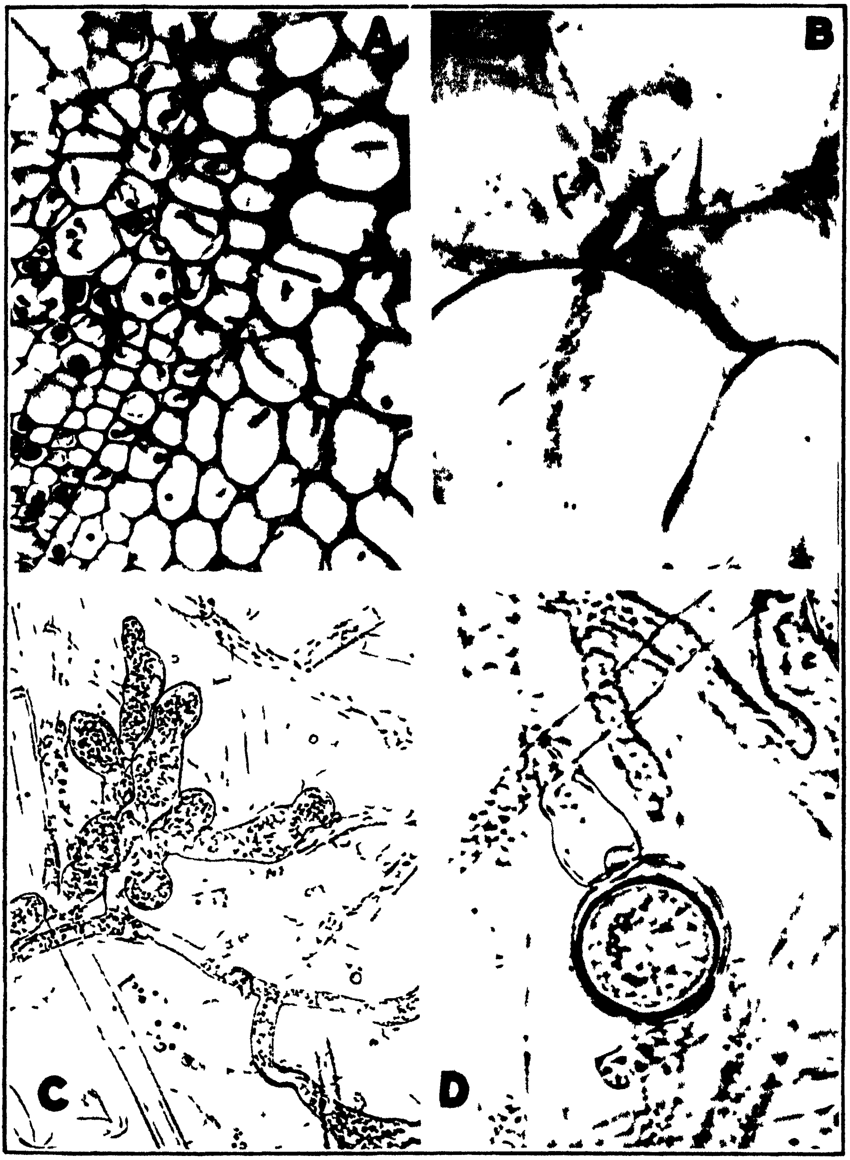

FIG. 2 A Section through a vascular bundle of an infected root Both the vessels and parenchyma show the presence of hyphae of *Pythium butleri* B Hypha of the parasite showing constriction at the point of cell wall penetration C The elongated and lobulate sporangium of the fungus D Antheridium and oogonium of *P. butleri*

and dying of the lower leaves of the plant that finally involved nearly all of the aerial parts. Affected leaves showed a brown, shrunken area extending from the base of the petiole toward the blade. Infected roots showed externally a deep brown discoloration, those portions nearest the crown being darkest, the color graduating toward the tip to a lighter shade, giving a dull mottled effect (Fig. 1, A) Internally, the roots showed a mummy-

brown discoloration (5) of those tissues attacked earliest. This discoloration graded off through a Dresden brown-Naples yellow to a Naples yellow in more recently invaded tissues (Fig. 1, B). The rot was of neither a soft nor a dry nature, the infected portions being relatively firm. Platings from the interior of affected roots yielded a pythiaceous organism, the pathogenicity of which was established by inoculations of and reisolations from numerous clean, injured and noninjured mature field-beet roots. In addition to the rotting of mature beet roots, the fungus was found capable of causing a severe damping off of sugar-beet seedlings. It was interesting to note that the virulence of the organism rapidly decreased in culture.

A histological study of affected root tissue showed the hyphae of the pathogen in profusion in all parenchymatous tissues and also in the vessels (Fig. 2, A and B).

Oogonia and antheridia of the fungus were produced readily when small pieces of artificially inoculated beet-root tissues were placed in dishes containing water blanks or Petri's solution (Fig. 2, D). Sporangia also were produced in this fashion, although they were more difficult to find than the former structures (Fig. 2, C). The best medium for sporangial production was found to be water blanks to which a small amount of greenhouse loam soil had been added prior to autoclaving. Oogonia from 48-hour-old water cultures averaged 24 μ in diameter, while the oospores averaged 20.5 μ. The organism was tentatively identified as *Pythium aphanidermatum* (Eds.) Fitzpatrick and cultures were sent to Dr. Charles Drechsler, who kindly consented to examine them. It is Dr. Drechsler's opinion that the fungus is one of the type generally referred to as *P. aphanidermatum,* but he considers that the binomial *P. butleri* is the proper one for the species in question on grounds set forth in an abstract in PHYTOPATHOLOGY (1).

Miestinger *et al.* (4) mention a root rot of beet in Austria caused to a lesser extent by *Pythium aphanidermatum,* and Simmonds (6), in 1933, reported the occurrence for the first time of a black rot of beet root in Australia induced by the same pathogen. Earlier, Edson (2) in this country, reported a fungus now generally considered as *Pythium aphanidermatum* (Eds.) Fitzpatrick, as a causal agent of damping off of sugar beet seedlings and capable of inducing injury to the feeding roots of half grown beets. No mention was made, however, of injury to the tap root proper. In his second paper (3) he described the organism as a new genus and species, *Rheosporangium aphanidermatus* Edson. Irrespective of whether or not the Rocky Ford fungus is considered as identical with the one previously described by Edson, the disease reported herein differs materially from that which he mentions.

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4. MIESTINGER, K., R. FISCHER, O. WATZL, and L. PORSCH. Wichtige Schädlinge und Krankheiten der Rübe in Österreich Bauernschr. 37. Niederösterr. Landes-Landwirtschafts-kammer. 28 pp. 1932.
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THE CONIDIAL STAGE OF HYPOXYLON PRUINATUM[1]

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(Accepted for publication March 7, 1938)

Povah[2] in 1924 was the first to describe the cankers caused by *Hypoxylon pruinatum* (Klotsche) Cke. and to suggest that the fungus was an important parasite on forest poplars. Lorenz and Christensen[3] in 1937 determined the amount of infection of *Populus tremuloides* by *H. pruinatum* in sample plots taken at random in the Lake States, and they state that "*Hypoxylon pruinatum* causes heavy losses in aspen throughout the Lake States and is probably one of the most important diseases of aspen throughout the range of the tree."

Despite the obvious importance of Hypoxylon canker, the organism has not been studied thoroughly. The perithecial stage has been known for some time, and the production of conidia by the fungus in culture has been observed, but the asexual spores produced in nature have not been described previously. A review of literature indicates that the conidial fructifications have been observed by several workers, but their true nature was not recognized.

Ellis,[4] in his description of a specimen collected in Iowa, states: "Surrounding the stromata and standing out obliquely like a coarse fringe, are short, coarse, bristle-like teeth, like the teeth of a Hydnum or Irpex. This curious growth also arises from the surface of the inner bark, for some distance around the stromata, soon throwing off the epidermis, and leaving the blackened surface of the inner bark exposed." On the basis of this character, he considered the fungus a new species and named it *Hypoxylon holwayii* Ellis. Ellis and Everhart[5] later decided that this fungus was within the range of variability of *H. pruinatum*. At this time they state: "The conidiiferous growth around the stromata may be only accidental, as it was not found in all the specimens."

[1] Paper No. 1617 of the Scientific Journal Series, Minnesota Agr. Exp. Station.
[2] Povah, A. Hypoxylon poplar canker. Phytopath. 14: 140–145. 1924.
[3] Lorenz, R. C., and C. M. Christensen. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. Mimeographed publication by the Bureau of Plant Industry, U.S.D.A. Washington, D. C., October 7, 1937.
[4] Ellis, J. B. General Notes: Botany. The Amer. Nat. 17: 192–196. 1883.
[5] Ellis, J. B., and B. M. Everhart. The North American Pyrenomycetes. P. 639. Newfield, New Jersey. 1892.

Povah[6] also may have seen conidial fructifications, as may be seen from his following statement: "In our specimens the bristly appearance is found commonly but not constantly. In some cases it is due to the presence of small perithecia protruding from the inner bark in which they are formed, little or no stroma being present. Sometimes the same spine-like appearance is obtained by the breaking down of the perithecia, leaving the jagged remnants of stroma and perithecial walls. In still other cases the dark teeth proved to be the more persistent parts of the inner bark left projecting after the softer part has been decayed."

The following account of the nature and development of the conidial stage of *Hypoxylon pruinatum* is based upon examination of material from numerous cankers developed naturally in the forest and of those produced as a result of artificial inoculations with cultures derived from ascospores and from conidia.

When the living bark of aspen trees is inoculated with *Hypoxylon pruinatum,* either naturally or artificially, the invaded cortex and the periderm above it first become light yellow. In a few weeks, due to shrinkage of the cortex, the cankered area is sunken below the general level of the bark and is split by many longitudinal shrinkage fissures; by this time the cortex has been partially destroyed and replaced by the black mycelium of the

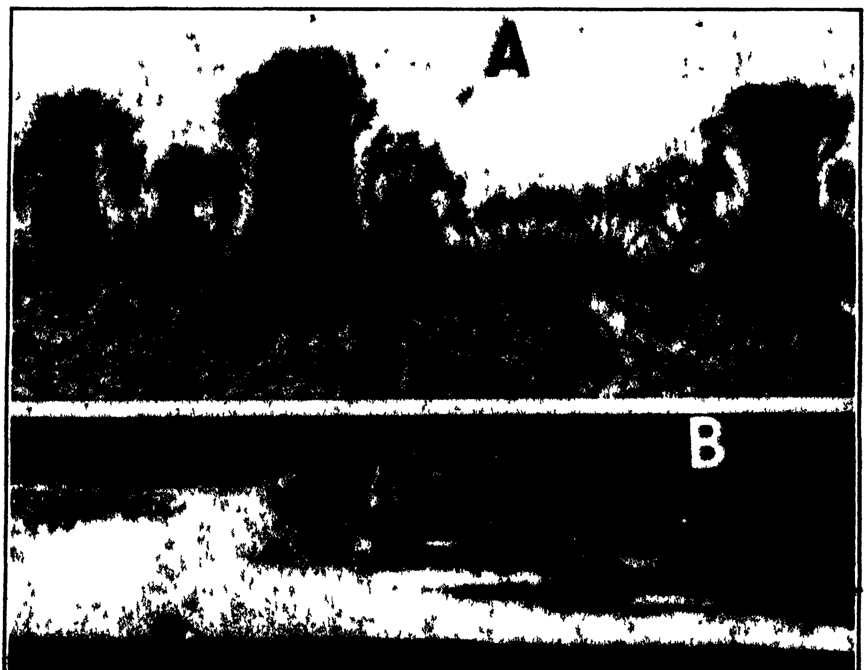

FIG. 1. A. Early stage in development of conidial stromata of *Hypoxylon pruinatum* on canker from natural infection. About × 85. B. Longitudinal section through cortex, showing group of old conidial stromata of *H. pruinatum* at the margin of an old canker. About × 15.

[6] See footnote 2.

fungus, which forms a subiculum on the surface of the cortex. About 3 months after infection the conidial fructifications begin to appear on this subiculum, still covered by the unbroken periderm. At first they are composed merely of rather compact, coremium-like tufts of branched conidiophores, as illustrated in figure 1, A, but they continue to increase in diameter and length and become more compact and stromatic in texture. The tips push out against the dead, thin, and brittle periderm, finally rupturing it and exposing the conidia. These stromata invariably have failed to develop on portions of the subiculum exposed by removing the periderm.

The bristly appearance of the conidial stromata is shown in figure 1, B. Due to weathering, and perhaps other causes, the stromata soon fall off, leaving only minute, jagged remnants and small circular scars that are clearly visible under a hand lens or binocular microscope, even on the surface of old cankers where perithecial stromata are present. The surface from which conidial stromata had been rubbed intentionally was compared with the surface of an old canker that bore the remains and scars of conidial stromata, and the appearance of the two was almost identical.

Two of the numerous cankers developed from inoculations made in June, 1937, were examined in October, 1937, and conidial stromata were found on one of them. They also were found on the borders of 15 3-year-old cankers developed from artificial inoculations on aspen trees at Cloquet, Minnesota, and on numerous older cankers of natural origin. By the time the perithecial stromata form, most, if not all, of the conidial fructifications have disappeared, and one could hardly recognize their remains without having seen them in a fresh and unbroken condition; hence it is not surprising that they have not been described previously.

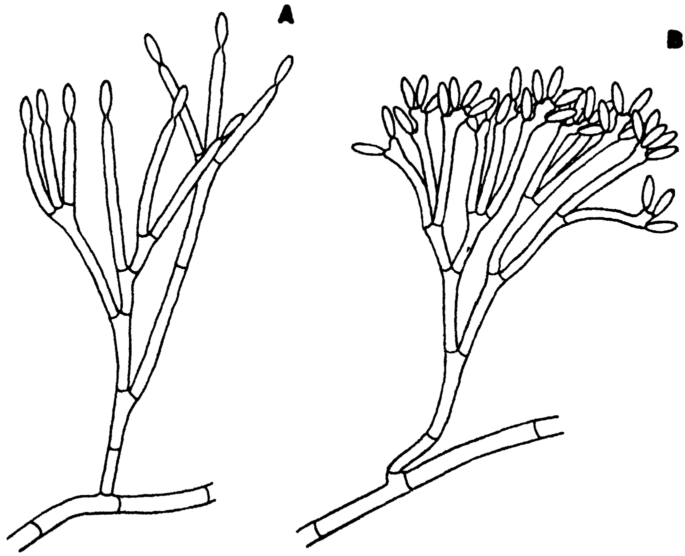

FIG. 2. A. Young conidiophore of *Hypoxylon pruinatum* in single-ascospore culture on malt agar. B. Old conidiophore of the same fungus.

The conidial stromata vary in basal diameter from 0.2 to 1.5 mm. and in length from 0.5 to 2.5 mm., averaging approximately 0.85×1.5 mm. The apex of a young stroma may be wider than the base, but the mature stromata always taper from the base toward the apex. The apices of those responsible for breaking the periderm are devoid of conidiophores, but, with this exception, conidiophores arise from the entire surface of both young and old stromata and of the subiculum. The conidiophores are 75 to 150 μ long, compound, the primary and secondary hyphae dichotomously branched, with either 2 or 3 terminal, spore-producing branches. At first, each branch bears a single terminal spore; but, later, the ends of branches are geniculate (Fig. 2, A and B). The conidia are hyaline, unicellular, fusiform to elliptical, measuring from 2×5.5–3×6.7 μ. They germinate readily on malt agar and produce one to several germ tubes. Cultures from conidia are within the range of variability of those from ascospores of *Hypoxylon pruinatum.*

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PHYTOPATHOLOGICAL NOTES

A Simple Method of Inoculating Wheat Seedlings With Paired Monosporidial Lines of Tilletia tritici and T. levis.—Studies of hybridization in *Tilletia tritici* (Bjerk.) and Wint. and *T. levis* Kühn necessitate the inoculation of wheat seedlings with paired monosporidial lines. The method described by Flor[1] has served the purpose thus far. He inserted the mycelium of 2 monosporidial lines into a wound at the base of each seedling. Hanna[2] and Becker,[3] likewise, used this method, and Becker stated that it was the best of 3 methods he tested. The writer[4] also used Flor's method but found it both tedious and time consuming, which limits its use for extensive inoculations. A more satisfactory method, since devised, is described below. This method is based on the principle that the secondary sporidia of *T. tritici* and *T. levis* are abjected forcibly from the sterigmata, as described by Buller and Vanterpool.[5] These workers used the same principle to inoculate seedlings with mass cultures.

A small piece of mycelium from each of the two monosporidial lines to be combined is transferred to a Petri dish containing potato-dextrose agar (1.5 to 2 per cent agar plus 1 per cent dextrose). The pieces of mycelium are

[1] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. [U. S.]. 44: 49–58. 1932.

[2] Hanna, W. F. The physiology of the fungi causing bunt of wheat. Proc. Fifth Pacific Sci. Congr., pp. 3195–3204. 1934.

[3] Becker, Theodor. Untersuchungen über Sexualität bei *Tilletia tritici* (Bjerk.) Wint. im Rahmen der Immunitätszüchtung. Phytopath. Zeitschr. 9: 187–228. 1936.

[4] Holton, C. S. A new pathogenically distinct race derived from a cross between *Tilletia tritici* and *T. levis*. Phytopath. 28: 371–372. 1938.

[5] Buller, A. H. R., and T. C. Vanterpool. The violent discharge of the basidiospores (secondary conidia) of *Tilletia tritici*. Buller, Researches in Fungi Vol. 5, 416 pp. Longman, Green and Co., New York. 1933.

planted on the surface of the medium near the edge of the dish, which is kept in an upright position until growth of the mycelium of both transfers has begun. The Petri dish is then turned on edge, with the side of the dish at which the mycelial colonies are growing at the top, and incubated in this position at a temperature suitable for development of the mycelium and secondary sporidia (10° to 18° C.). As growth continues the secondary sporidia, being forcibly abjected, fall downward, come to rest on the agar surface, and germinate to form mycelium, which produces more sporidia. Thus, a part of the agar surface becomes covered with sporidia and mycelial growth of the two cultures, as shown in figure 1. The culture is then ready

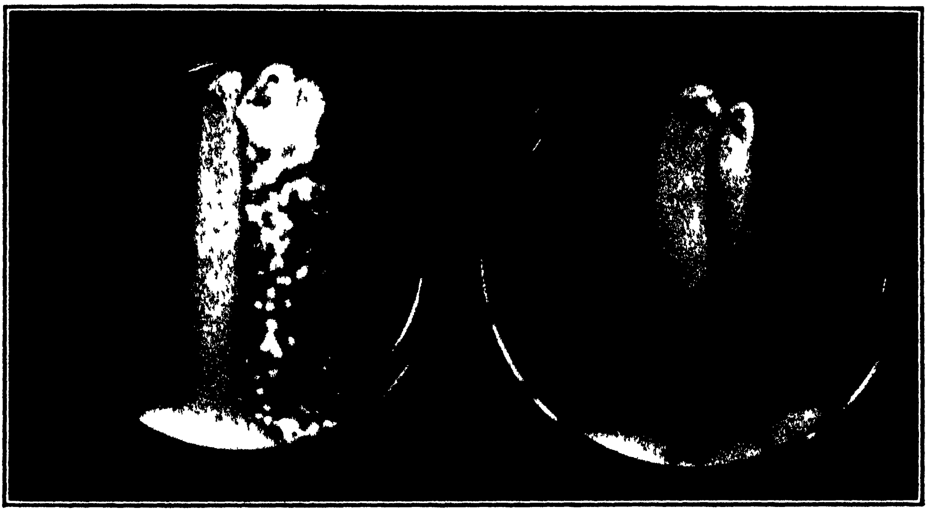


FIG. 1. Paired monosporidial lines of *Tilletia tritici* and *T. levis*, which were grown for inoculation purposes. Both pairs produced infection.

for use in inoculating the seedlings, and the Petri dish may be laid flat. The development of these cultures may require 2 to 3 weeks, depending upon the rate of growth of the monosporidial lines used. Greater coverage of the agar surface and a better mixture of the sporidia and mycelia of the two lines, than those shown in figure 1, may be obtained by transferring several pieces of mycelium of the two lines to one plate or by shifting the plates each day so that the sporidia spread in several directions instead of one.

The Petri-dish cover is then removed and inverted and the dish containing the culture also is inverted. Sterile filter paper is placed on the inside of the cover and moistened with sterile water. Fifty to 100 surface-disinfected kernels of a susceptible wheat variety are so placed on the moist filter paper as to be directly beneath the culture, when the inverted dish is put into the cover. The inverted Petri dish is incubated at 10° C. for 10 to 14 days, depending on the rate of growth of the seedlings. During this period secondary sporidia fall on the seedlings and germinate, thus exposing the seedlings to infection. When the seedlings grow larger they come into contact

with the sporidia and mycelium of the monosporidial lines above them, being thus further exposed to infection. The filter paper must be kept moist during the incubation period by adding sterile water from time to time. At the end of the incubation period the seedlings are transplanted to the greenhouse or field, either individually or as a group. The best results are obtained when the filter paper bearing the seedlings is lifted out of the Petri dish and all of the seedlings are transplanted in a group, still attached to the filter paper, without disturbing the roots, which is unavoidable when the seedlings are transplanted individually.

This method has the obvious advantage of permitting the testing of a large number of sporidial pairs and the inoculation of many seedlings in a short time without mechanical injury to the seedlings. Care must be exercised, however, to avoid interference by contaminating organisms while growing the inoculum and inoculating the seedlings. The gnarled and twisted seedlings are difficult to transplant, but this may be partly overcome by removing for a day the upper half of the inverted Petri dish before transplanting, to allow the seedlings to become erect.

Three experiments have been conducted in the last 2 years in which the inoculations were made by the above method, and high infection percentages were obtained each time. In the most extensive series of these inoculations, 26 of 74 combinations of monosporidial lines produced infections ranging from 1 to 57 per cent, with the majority above 20 per cent. It is very probable that still higher percentages of infection may be obtained with added experience and improved technique.—C. S. HOLTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating with the Washington and Idaho Agricultural Experiment Stations.

A Heritable Abnormality of Beans Resembling Mosaic.[1]—A leaf variegation in beans (*Phaseolus vulgaris* L.), the symptoms of which somewhat resemble a virus disease, was observed during an investigation of mosaic resistance. The symptoms differ from those produced by the common bean mosaic virus. Inoculation studies proved that this variegation was not caused by an infectious virus. Harrison and Burkholder[2] and Horsfall *et al.*[3] reported having found in the Wisconsin Refugee, a variety resistant to the common bean mosaic, plants that were apparently affected with a new virus disease. From the descriptions published by those workers, it is possible that the abnormal plants they observed are identical with the chlorophyl-

[1] Appreciation is expressed to F. J. Stevenson who offered valuable suggestions during the progress of the work and who assisted in the interpretation of the results, and to C. F. Andrus, who kindly permitted the use of certain data that were obtained from a number of segregating progenies used by him in a genetic study of bean anthracnose.

[2] Harrison, A. L. and W. H. Burkholder. U. S. Dept. Agr. Bur. Plant Indust. Plant Disease Reporter 20: 290–291. Oct. 15, 1936.

[3] Horsfall, J. G., W. H. Burkholder and O. A. Reinking. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reporter 21: 318–319. Sept. 15, 1937.

deficient plants reported here. They are different from the two chlorophyl-deficient types reported by Parker.[4,5]

Such plants were noted principally in hybrids in which Corbett Refugee was used as either of the parents. A study of a number of these hybrids by the writer indicated that the variegation was a heritable character. This character appeared in every varietal combination with Corbett Refugee but not in 100 per cent of all crosses involving each combination. This is evidence that Corbett Refugee is a mixture of biotypes.

The symptoms of this variegation vary considerably. The primary leaves may be practically devoid of chlorophyl and in such instances they frequently die. In other cases islands of green or streaks of yellow appear on the leaves. Such plants occasionally grow to maturity with the production of a few pods. Sometimes a plant may not show this defect until it has attained considerable size and then only a small amount of variegation is noted and on only a few leaves. Such plants grow to normal size.

When extremely variegated plants grow beyond the seedling stage, they are always stunted, with distorted leaves and pods and shortened internodes. They may also be rosetted. Very frequently defective plants manifest the variegation on only one-half of each leaflet accompanied by a curling of the affected leaflet toward the variegated portion, which may be the result of continuous growth of the unaffected cells accompanied by almost complete cessation of growth in the affected area. Pods produced on extremely affected plants are often curled, distorted, and smaller than normal.

The F_1 plants from reciprocal crosses of Corbett Refugee, which carries the gene for variegation, by other normal green plants of several varieties were green. The genetic constitution of Corbett Refugee is not entirely known. In the F_2 generation, a ratio of 15 normal green to 1 variegated plant was obtained from 6729 plants. The deviation from the 15 to 1 ratio was 0.46 which is 0.31 times the standard error. The reciprocal crosses in every case behaved alike.

The F_3 progenies of the green F_2 plants segregated into 3 classes, all green, 15 green to 1 variegated; and 3 green to 1 variegated. These three classes were approximately in the ratio of 7:4:4, respectively. The value of X^2 is 5.68 and P equals 5.8. The true breeding green progenies were slightly deficient while the segregating progenies were slightly in excess of the normal distribution. The variegated plants found in the F_2 generation did not breed true, a phenomenon that cannot be accounted for at present. Further studies of this variegated character and its genetic relationships are being conducted in an attempt to clarify the points in question.

Although the genetic hypothesis of duplicate genes does not explain all of the facts, the results show definitely that the abnormality is heritable and

[4] Parker, M. C. The inheritance of a yellow-spot character in the bean. Jour. of Heredity 24: 481–486. December, 1933.

[5] ———. Inheritance of a leaf variegation in the common bean. Jour. of Heredity 25: 165–170. April, 1934.

not the result of a virus infection.—W. J. ZAUMEYER, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

A New Microcyclic Coleosporium on Limber and Piñon Pines.[1]—In the summer of 1937 Ivan H. Crowell collected an orange-red needle rust on *Pinus flexilis* and *P. edulis* in southern New Mexico. Macroscopically, the rust (Fig. 1, A) has the appearance of the peridermia of species of the genus *Coleosporium.* Microscopic examination of free-hand sections demonstrated, however, that the apparent peridermia were actually gelatinous telial horns composed of chains of teliospores (Fig. 1, B). The teliospores germinate at

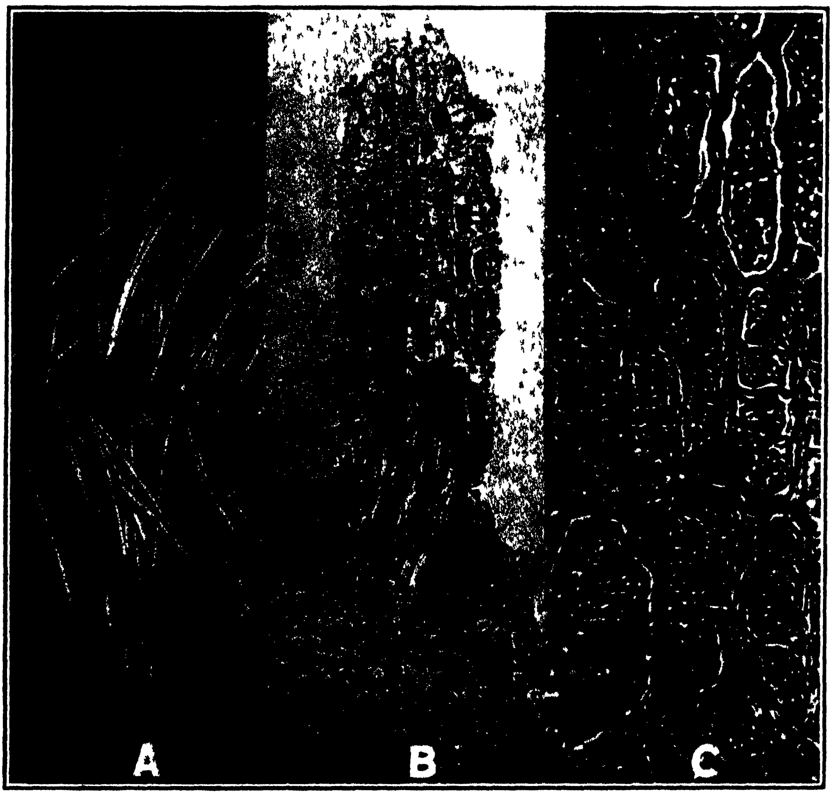


FIG. 1. *Coleosporium crowellii* on *Pinus.* A. Telial horns on *Pinus flexilis.* Approximately × 1. Photograph by Crowell. B. Free-hand section of a telium on *Pinus edulis* showing teliospores imbedded in the gelatinous matrix. The apical spores had germinated and disintegrated, the median spores were in process of germination, while the basal spores were unicellular. × 110. C. Magnified portion of B, showing the internal basidium. Sterigmata and basidiospores had not yet developed. × 510.

once with the formation of an internal basidium (Fig. 1, C). This immediate germination empties the spores in the upper one-half or one-third of the telial horn, so that the base is orange colored, while the apex is nearly colorless.

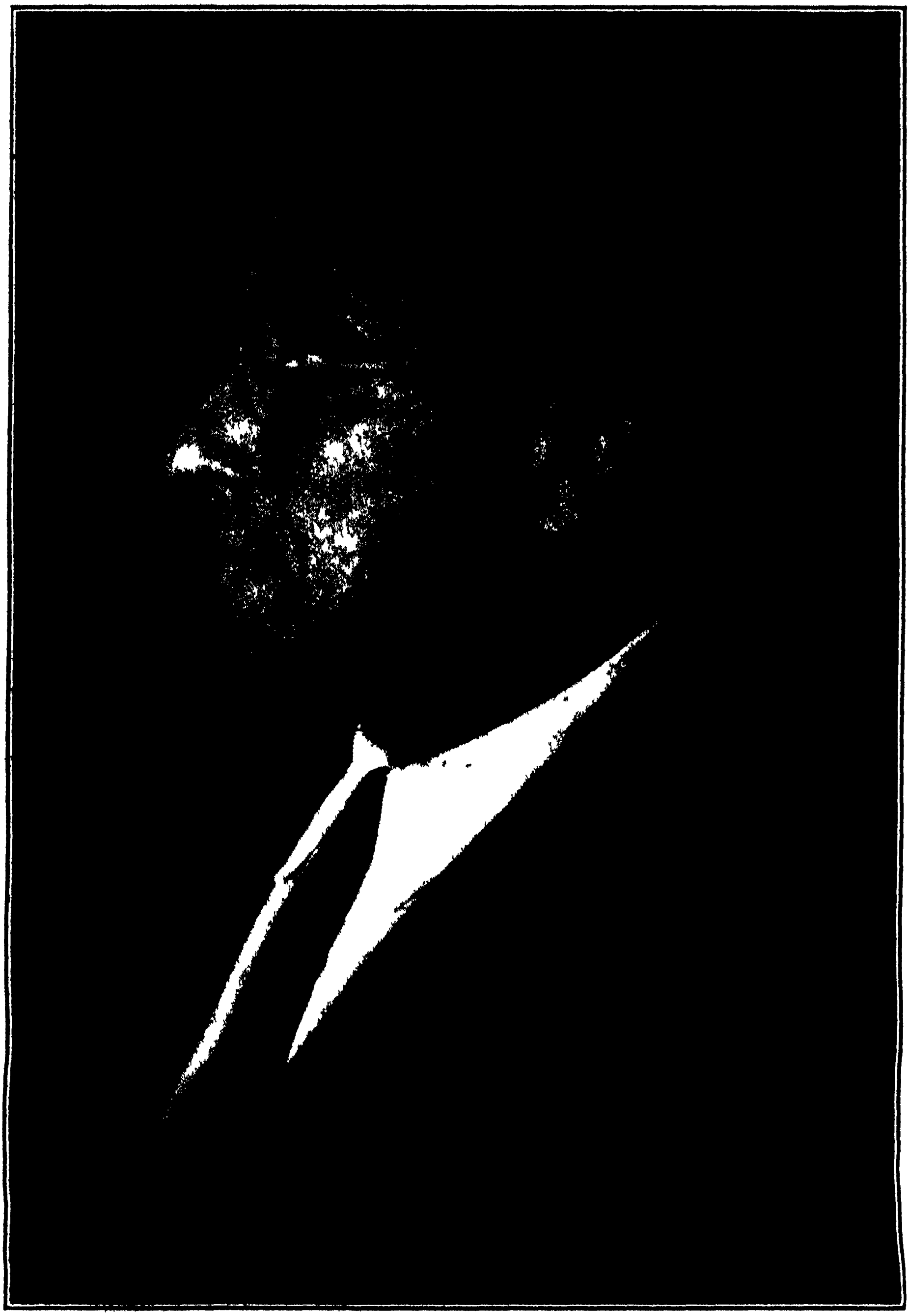
[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

The rust is considered to be a microcyclic species of the genus *Coleosporium,* strongly simulating the aecial habit in size of telia and catenulation of spores, and it inhabits the genus *Pinus,* aecial host of the macrocyclic species of *Coleosporium.* A formal description of this rust, here named in honor of Dr. Crowell, follows.

Coleosporium crowellii, sp. nov. Pycniis non visis. Teliis amphigenis, subepidermalibus, gelatinosis, linguaeformibus, 0.3–0.7 mm, alt., 0.5–2 mm, lat., 0.1–0.15 mm, cr. (*Pini edulis*), 0.5–1.0 mm, alt., 0.1–0.3 mm, lat., 0.1–0.15 mm cr. (*Pini flexilis*) primo aurantiacis dein pallide flavis vel albido-flavis; teliosporis in catenas, cylindraceis, 17–24 × 50–65 μ, primo 1-cellularibus, dein ob germinationem 4-cellularibus, levibus, intus aurantiacis; basidiosporis globosis 14–18 × 16–20 μ. In foliis *Pini edulis* Engelm., Cloudcroft, Otero Co., N. M., July 3, 1937; Kingston and Hillsboro, Sierra Co., N. M., July 9, 1937; Wrights Cabin, Grant Co., N. M., July 9, 1937, *Ivan H. Crowell.* In foliis *Pini flexilis* James, James Canyon, Otero Co., N. M., Aug. 23, 1937, *Ivan H. Crowell* (Type). Type deposited in the Arthur Herbarium, Purdue University Agricultural Experiment Station.

The difference in the size of the telia from the two hosts is conspicuous, but in the absence of other differential characters, is not considered sufficient basis for a separation of the two collections.—GEORGE B. CUMMINS, Purdue University, Lafayette, Indiana.

Septoria brevispora (Sacc.) Zeller renamed.—The above name is untenable since Ellis and Davis used the same combination for a *Septoria* parasitic on *Bromus ciliatus* (Trans. Wisc. Acad. p. 99. 1903). I am therefore designating the *Septoria* on *Rubus* as **Septoria darrowii,** n. nom. (Syn. *S. brevispora* (Sacc.) Zeller) (Phytopath. **27**: 1002. 1937), in respect to Dr. George M. Darrow, U. S. Department of Agriculture.—S. M. ZELLER.



JACOB JOSEPH TAUBENHAUS
1885–1937

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B. YOUNGBLOOD

Dr. Jacob Joseph Taubenhaus, for nearly 22 years Chief of the Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, and a charter member of the American Phytopathological Society, after an illness of about five months, died of heart trouble, terminating in pneumonia, on December 13, 1937.

Dr. Taubenhaus was born at Safed, Palestine, near the Sea of Galilee, October 20, 1885. His interest in agriculture began in his very early childhood. At twelve years of age, he entered the only agricultural school then in Palestine, at Mikve, Israel, conducted by the Alliance Israelite Universelle. Upon completion of his work there in 1898, he came to the United States and entered the National Farm School at Doylestown, Pennsylvania, from which he was graduated in 1904. At this School, he showed his deep interest in horticulture, which continued the rest of his life. He entered Cornell University in 1904, where he was graduated with the degree of Bachelor of Science in 1908 and Master of Science in 1909, in plant pathology and physiology. He next enrolled at the University of Pennsylvania, where he studied under Dr. J. W. Harshberger and was granted the degree of Doctor of Philosophy in 1913. His thesis, submitted in partial fulfilment, was entitled "The Diseases of the Sweet Pea."

Young Taubenhaus was ever an earnest and diligent worker. At Cornell, he was sustained by student labor, and his graduate work there was made possible by a personal loan from Dean Liberty Hyde Bailey, to whom Taubenhaus was very devoted. During his last year at Cornell he served as landscape gardener to President Andrew D. White, retired. As an undergraduate, he carried on experiments to determine the effect of ether on seed germination. He was also strongly influenced by Dr. H. H. Whetzel, under whom he became interested in the diseases of ornamental plants.

To Taubenhaus, the United States was a land of opportunity. He was fond of our economic and social institutions and of our form of government, all of which he studied closely. He became a naturalized citizen of the United States in 1908.

He became Assistant Plant Pathologist at the Delaware Agricultural Experiment Station in 1909, and, in 1913, was promoted to the rank of Associate, which position he held until 1916. Here, Taubenhaus collaborated with Dr. Melville T. Cook in the study of the possible relations of tannins to resistance to plant diseases and carried on extensive studies of the diseases of sweet potatoes. A noteworthy contribution at the time was his description of *Sclerotium bataticola* Taub.

Dr. Taubenhaus became Chief of the Division of Plant Pathology and Physiology at the Texas Agricultural Experiment Station in 1916. This position he held until the time of his death. From 1924 to 1937, he was also a member of the graduate faculty of the College.

For some years after going to the Texas Station, he did pioneer work in arousing interest in disease control. In this connection, he identified diseases and made recommendations for control to the Extension Service and identified the causes of loss in transit for the railroads. Despite these services, he carried on extensive investigations, rarely interrupted even by vacations. While devoting major attention to the study of the cotton root rot (*Phymatotrichum omnivorum*), he continued his interest in the diseases of sweet potatoes and of many other plants, particularly onions, melons, and tomatoes.

The Phymatotrichum disease study was long continued and encyclopedic. His first and most extensive publication on this subject, 1923, advanced the idea that the puzzling differences between this disease and the Fusarium and other root diseases are to be explained by the close association of living mycelium of the fungus with the roots—particularly, with partially decayed, as compared to completely decayed portions of infected roots—and by the fact that the spread of the disease is chiefly along roots rather than through the soil itself. He developed methods of inoculating plants with this disease-producing organism on a large scale, which facilitated field experiments. Inoculation experiments with monocotyledonous plants verified the conclusion that such plants are immune from the *Phymatotrichum* fungus. This fact is basic to the control measures now advocated in Texas, including sorghum in crop rotation and "sorghum barriers," which latter tend to block the advance of the disease across cotton fields. The results of his extensive studies on the host range of the Phymatotrichum root rot were embodied in a bulletin, which was completed only a few months before his final illness. He and his associates tested the relative susceptibility of more than 2,000 species and varieties of flowering plants to the Phymatotrichum root-rot fungus.

In Texas he worked with many other specialists, State and Federal. Beginning in 1927, large State appropriations for the expansion of root-rot research made possible the employment of a number of associates. In addition, he cooperated with a number of agronomists and chemists of the Texas Station in an endeavor to follow out the varied ramifications of the root-rot problem.

Dr. Taubenhaus was Russell Lecturer for the Massachusetts State Horticultural Society in 1916; and, was chairman of the committee on the preparation of exhibit entitled "The Diseases of Plants," for the Texas Centennial Central Exposition, 1936.

Dr. Taubenhaus' life was characterized by the great zeal with which he conducted his research and the large scale upon which he planned many of his experiments. Instead of using a few cultures, he used hundreds of them.

Some who knew him intimately feel that too long and continuous application to his work may have hastened his death.

Dr. Taubenhaus was a charter member of the American Phytopathological Society and had been secretary of the Southern Division; Fellow, American Association for the Advancement of Science; Texas Academy of Science (chairman, "Grants" committee); member, Botanical Society of America, Mycological Society of America, American Rose Society, Natural History Society of Delaware, Nature Association, National Association of Gardeners, Authors' League of America, Authors' Club (London), Cotton Disease Council (chairman of section on Phymatotrichum root rot); was a member of the Sigma Xi fraternity; Mason; and B'nai B'rith.

In addition to his scientific work, he took an active part in religious activities. For years he was leader of the congregation of Temple Freda in Bryan, Texas. He also served as president of the local lodge of B'nai B'rith there. On the college campus, he organized the Hillel Club composed of Jewish students and acted as their guide and adviser. During the influenza epidemic of 1918, he spent all of his spare time in the hospital aiding in nursing. In addition, he was a member of the Provisional Executive Committee for Zionist affairs.

Dr. Taubenhaus was a director for Texas, and a constant supporter, of the National Farm School, Doylestown, Pa. In 1926 he was the principal speaker and guest of honor at a banquet given in New York City for the benefit of the National Farm School at which 1,000 guests paid $1,000 per plate, the proceeds forming a million dollar endowment fund for the school.

Dr. Taubenhaus married Esther Hirschenson of Hoboken, New Jersey, in 1910—children, Leon Jahir and Ruth Ziporah. In addition to his immediate family, he is survived by his father and mother, Meyer and Bath-sheba Taubenhaus, three brothers and three sisters in Haifa, Palestine, and another brother in New York City.

His untimely death was a great loss to the State of Texas; and his stimulating presence will be greatly missed from the annual meetings of The American Phytopathological Society. He practically always attended these meetings and participated in the programs.

Dr. Taubenhaus was a prolific writer. Because of space limitations only his principal publications are listed below. Besides these, Dr. Taubenhaus, or he and his associates, also wrote on a wide range of subjects for professional and agricultural journals. He presented many papers on plant diseases before farmers' short courses, local and national agricultural and scientific societies, and was a frequent speaker over the college radio station, WTAW.

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(List assembled by Dr. W. N. Ezekiel)

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5. The relation of parasitic fungi to the contents of the cells of the host plants (II. The toxicity of vegetable acids and the oxidizing enzymes)—(Cook, Mel T., and J. J. Taubenhaus). Del. Agr. Expt. Sta. Bul. 97. 1912.
6. A further study of some *Gloeosporiums* and their relation to a sweet pea disease. Phytopath. 2: 153–160. 1912.
7. The black rots of the sweet potato. Phytopath. 3: 159–166. 1913.
8. Recent studies of some new or little known diseases of the sweet potato. Phytopath. 4: 306–320. 1914.
9. The diseases of the sweet pea. Del. Agr. Exp. Sta. Bul. 93. 1914.
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19. A text-book of mycology and plant pathology, by John W. Harshberger. (A review). Sci. (n.s.) 48: 516–517. 1918.
20. Diseases of truck crops and their control. E. P. Dutton Co., New York. 1918.
21. Pink root of onions. Sci. (n.s) 49: 217–218. 1919.
22. Field diseases of the sweet potato in Texas. Texas Agr. Exp. Sta. Bul. 249. 1919.
23. Storage and diseases of the sweet potato in Texas. Texas Agr. Exp. Sta. Bul. 250. 1919.
24. Recent studies on *Sclerotium rolfsii* Sacc. Jour. Agr. Res. [U. S.] 18: 127–138. 1919.
25. Diseases of greenhouse crops and their control. E. P. Dutton Co., New York. 1920.
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29. On a peculiar disease of mulberry fruit. Nature-study Review. 282–285. (1921?)
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50. A disease of young cotton plants caused by *Sclerotium rolfsii*. (Ezekiel, Walter N., and J. J. Taubenhaus). Phytopath. **21**: 1191–1194. 1931.
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(Several further papers on which Dr. Taubenhaus' name will appear are to be prepared from work completed or partly completed before his death).

STUDIES ON THE NUTRITION OF FUNGI

I. THIAMIN, ITS CONSTITUENTS, AND THE SOURCE OF NITROGEN[1]

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Fungi other than obligate parasites may be divided into 2 groups: those that manufacture their own auxithals (growth-promoting substances), and those that must secure these from extraneous sources. The first group grows readily on media composed of the essential inorganic salts and of dextrose, while the second group fails to grow on such media, regardless of time or environment, but must have, in addition, other substances. The identification of such substances constitutes the purpose of this series of papers.

Yeast and yeast extract contain auxithals for bacteria, fungi, higher plants, and animals. In addition to some of the known vitamins, of amino acids, other organic nitrogenous compounds, organic acids, carbohydrates, fats, and mineral salts, yeast contains a number of chemically unidentified, but physiologically active, "factors" that have been studied by animal physiologists; but it is not yet known if these factors are involved in the growth of fungi. The reader is referred to Lewis (11), Lecoq (9), and Von Euler (4) for a comprehensive review of these "factors," as well as of vitamins.

Outstanding among the known compounds of the yeast are the members of the Vitamin B complex and amino acids. The inorganic constituents are not considered here because the ash of yeast has been found to be without auxithallic properties. The carbohydrates also are excluded because all of our test organisms are capable of utilizing dextrose, and the addition to the medium of the carbohydrates extracted from the yeast has failed to show any activity. Other substances known to be present in yeast, such as succinic acid, uracil, glutathione, and ether-soluble materials, were tested and found ineffective. Nor did nicotinic and pimelic acids show any auxithallic property for our fungi, although Knight (8) found nicotinic acid to be essential for *Staphylococcus aureus*, and Mueller (14) reported that pimelic acid in minute amounts stimulated the diphtheria bacillus. Pantothenic acid, with or without inositol, proved inactive and heteroauxin failed to show any stimulation. This narrowed the field down to the source of nitrogen and to accessory growth substances, particularly thiamin.

The source of nitrogen is an important limiting factor in the nutrition of fungi, and the use of the "wrong" kind of nitrogen compound may often lead the investigator astray. For a comprehensive review of nitrogen assimilation, the reader is referred to Robbins (17). But, even the "right"

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kind of nitrogen, or combinations of nitrogenous compounds, often may be ineffective without the presence of thiamin.

Students of fungi did not devote much attention to the effect of vitamins until recently. Lepeschkin (10) found that 0.001 per cent of vitamin B (the different fractions of this vitamin complex were not known then), added to a culture solution with ammonium sulphate as the only source of nitrogen, caused a more rapid development of *Saccharomyces cerevisiae* and *Penicillium glaucum*. This stimulation was observed only during the earlier stages of growth; eventually, no difference could be detected between the controls and the treated cultures. In this connection it is interesting to note that Richards (16) arrived at a similar conclusion concerning the effect of pantothenic acid on yeast. Williams and Saunders (34) report that thiamin exerted very little effect on many strains of yeast, but "Old Process" yeast was stimulated by doses of 0.008 gamma and upwards. It should be stressed here that a substance that does not actually induce growth, but merely brings about a temporary stimulation, cannot be considered as a vital factor in the metabolism of fungi but merely as a stimulant; it seems, however, that thiamin, unlike most stimulants, also acts as a vital accessory factor in the nutrition of some fungi, as can be seen by the work of Schopfer (23), who showed that even such a minute dose as 0.0005 gamma per ml. of medium induced growth in *Phycomyces blakesleeanus*. In the absence of this vitamin, there was only a slight and submerged growth, whereas, in its presence, aerial mycelium, sporangiophores, as well as zygospores, developed very readily. Some 13 different amino acids, used separately or combined, failed to induce growth in this fungus. Pure riboflavin (vitamin B_2) was without any effect. Burgeff (3) confirmed and extended Schopfer's findings. Schopfer (24) extended his investigations to other fungi and found that *Absidia ramosa, Parasitella simplex, Mucor ramannianus, Dicranophora fulva, Chaetocladium brefeldii, Choanephora cucurbitarum, C. persicaria,* and *Pilaira anomala* also were induced to grow in synthetic media under the influence of thiamin. However, some other fungi, such as species of *Rhizopus,* failed to respond and some were even inhibited by the vitamin. Searching for growth factors other than thiamin, Schopfer (25) tested urine, Aspergillus extract (26), and wheat germ (27). He found that the urine factor that influenced the growth of *Phycomyces blakesleeanus* was neither thiamin nor auxin. The Aspergillus extract exerted the same influence upon the test fungus as thiamin. From wheat germ he separated 2 factors: while the nontreated wheat-germ extract induced growth in both *P. blakesleeanus* and *Rhizopus suinus,* the filtrate resulting from the animal-charcoal treatment proved inactive for *Phycomyces* and active for *Rhizopus,* while the eluate was active for *Phycomyces* and inactive for *Rhizopus.* The factors responsible for the growth of *Phycomyces* are believed by him to be disintegration products of thiamin, ineffective for animals but effective for the fungus. Kögl and Fries (6) noted that thiamin was the growth factor required by *Phycomyces blakesleeanus, Phytophthora cactorum, Nectria coccinia, Sclerotinia cinerea,*

Polyporus adustus, P. abietinus and *Tricholoma nudum.* The vitamin proved active also for *Fomes pinicola, Trametes cinnabarina,* and *T. serialis,* although malt extract and yeast extract proved more beneficial. When thiamin, inositol, and biotin (7) were combined, *Nematospora gossypii* (*Ashbya gossypii*) showed the greatest activity; slight growth resulted when these 3 constituents were used separately; biotin and inositol proved active but not so effective as when thiamin was added. Biotin, inositol, and thiamin induced the greatest growth in *Lophodermium pinastri,* biotin and thiamin proved less active, whereas, used separately, they failed to induce any growth.

For a review of the effect of thiamin on microorganisms and on higher plants the reader is referred to Bonner (2).

THE EFFECT OF CRYSTALLINE THIAMIN

At first, some 25 representative fungi were used as test organisms. These do not make an appreciable growth on a medium consisting of 1 g. of ammonium nitrate, 0.5 g. each of dihydrogen potassium phosphate and magnesium sulphate, 5 g. of dextrose, 20 g. of Bacto agar, and 1,000 ml. of distilled water. The addition of 1 g. of Bacto yeast extract to this medium, however, enabled all the organisms to grow. When a minute quantity (1 part per 20 million) of either synthetic or natural thiamin was used instead of yeast extract, 10 fungi were induced to grow. When the medium was modified by substituting a mixture of amino acids[2] for ammonium nitrate, and without the presence of thiamin, only one organism, *Basidiobolus ranarum,* grew readily, the remaining 24 continuing to make slight or no growth. The addition of thiamin to this modified medium enabled 14 organisms to make a rich growth. The results are listed in table 1. All nutrient media used throughout this work were adjusted to pH 5.5 before tubing.

Obviously, yeast extract contains more than one auxithal because all 25 fungi grow in its presence, while only 14 of them grow in presence of thiamin. The thiamin content of Bacto yeast extract is given by the manufacturers as 5 Sherman units per gram; too small a quantity to support a maximum growth. *Coprinus lagopus, Phytophthora erythroseptica, Pythiomorpha oryzae,* and *Pythium polymastum* failed to show a luxuriant growth in the presence of 0.1 per cent yeast extract, but attained it when the mixture of amino acids and thiamin were added to the synthetic medium. In so far as the performance of the 14 organisms listed in the last column is concerned, a suitable source of nitrogen and thiamin are the substances that control growth. One additional organism, *Basidiobolus ranarum,* grows readily without the vitamin, provided that the source of nitrogen is suitable. This leaves 10 fungi that require something other than thiamin or any of the foregoing amino acids for growth.

Riboflavin is the only other member of the vitamin B complex available

[2] *d*-arginine, *d*-glutamic acid, *l*-aspartic acid in 2 parts each; *dl*-*a*-alanine and glycine 1 part each. 1 gram of this mixture was used in each 1,000 ml. of medium.

TABLE 1.—*The effect of crystalline thiamin on the growth of fungi*

Synthetic medium	Synthetic medium plus yeast extract 1000 ppm.	Synthetic medium plus thiamin 0.05 ppm.	Synthetic medium with amino acid mixture (0.1%) in place of ammonium nitrate; no thiamin	Synthetic medium with amino acid mixture and thiamin 0.05 ppm.
None grew	Good growth *Allomyces javanicus* *Ascobolus viridulus* *Ashbya gossypii* *Basidiobolus ranarum* *Blakesleea trispora* *Ceratostomella multi-annulata* *Chaetocladium brefeldii* *Collybia tuberosa* *Dipodascus uninucleatus* *Lentinus tigrinus* *Nyctalis asterophora* *Ophiobolus oryzinus* *Pleurotus corticatus* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pythium oligandrum* *Saprolegnia parasitica* *Sordaria fimicola* *Sporormia intermedii* *Thraustotheca clavata* Fair growth *Coprinus lagopus* *Pythiomorpha oryzae* *Phytophthora erythroseptica* *Pythium polymastum*	Good growth *Blakesleea trispora* *Collybia tuberosa* *Lentinus tigrinus* *Phycomyces nitens* *Phytophthora erythroseptica* *Pythimorpha gonapodioides* *Pythimorpha oryzae* *Pythium polymastum* Fair growth *Chaetocladium brefeldii* *Pythium oligandrum*	Good growth *Basidiobolus ranarum*	Good growth *Blakesleea trispora* *Collybia tuberosa* *Coprinus lagopus* *Chaetocladium brefeldii* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Phytophthora erythroseptica* *Pythiomorpha gonapodioides* *Pythiomorpha oryzae* *Pleurotus corticatus* *Pythium oligandrum* *Pythium polymastum*

commercially in the form of pure crystals. Added to the synthetic medium (1 ppm.) with ammonium nitrate as source of nitrogen, it failed to endow the medium with any auxithallic property; substitution of the amino acid mixture for ammonium nitrate proved only a little better, the stimulation being so slight as to be negligible.

A comparison of the third and last columns of table 1 shows that there might be a close relation between the activity of thiamin and the source of nitrogen. In order to demonstrate this more conclusively, 4 additional nitrogen compounds were used separately in place of the ammonium nitrate of the synthetic medium. In this and in all subsequent cases the amount of the nitrogen compounds used was 0.1 per cent.

The medium containing potassium cyanide was adjusted to pH 7 in order to prevent the production of hydrogen cyanide.

TABLE 2.—*The effect of different sources of nitrogen and thiamin upon growth*

Synthetic medium with sodium nitrate in place of ammonium nitrate and 0.05 ppm. thiamin	Sodium nitrite as source of nitrogen and 0.05 ppm. thiamin	Urea as source of nitrogen and 0.05 ppm. thiamin	Potassium cyanide as source of nitrogen and 0.05 ppm. thiamin
Good growth	Fair growth	Good growth	Good growth
Blakesleea trispora	*Blakesleea trispora*	*Coprinus lagopus*	*Lentinus tigrinus*
Collybia tuberosa		*Lentinus tigrinus*	*Pilaira moreaui*
Lentinus tigrinus		*Nyctalis asterophora*	*Pythium oligandrum*
Pythiomorpha gonapodioides		*Phycomyces nitens*	
		Pilaira moreaui	
Fair growth			Fair growth
Chaetocladium brefeldii		Fair growth	*Blakesleea trispora*
Phycomyces nitens		*Blakesleea trispora*	*Collybia tuberosa*
Pythiomorpha oryzae		*Phytophthora erythroseptica*	*Coprinus lagopus*
Pythium polymostum		*Pythiomorpha oryzae*	*Nyctalis asterophora*
			Phycomyces nitens

Table 2 supports the fact that thiamin is inactive unless the proper source of nitrogen is supplied. Sodium nitrate plus the vitamin induced growth in fewer organisms than ammonium nitrate because ammonium nitrogen can be utilized by more organisms than nitrate nitrogen. Sodium nitrite proved the poorest source of nitrogen; and, despite the presence of thiamin, only one organism, *Blakesleea trispora,* made a fair growth. It is doubtful if this fungus utilized nitrite nitrogen; the trace of nitrogen present in agar-agar was probably its only source of nitrogen. Urea was slightly more favorable than either sodium nitrate or potassium cyanide.

Agar-agar is freely used when solid media are desired; yet, it is a complex substance of unknown constitution. In order to determine the possible nutritional effect of agar-agar upon our organisms, 2 series of liquid cultures were prepared: the first series contained ammonium nitrate as the only source of nitrogen, while the second series received the amino acid mixture. Thiamin was added to both (0.05 ppm.). All 14 of the thiamin-responding organisms grew readily in the second series of solutions, while in the first series no or

only a slight growth was made by *Chaetocladium brefeldii, Collybia tuberosa, Pythiomorpha gonapodioides,* and *Pythium oligandrum.* Apparently, these fungi are unable to utilize ammonium nitrate without the presence of agar-agar. When nitrogen was left out of the medium, but the usual amount of the agar plus thiamin added, a fair growth was made by the foregoing as well as other organisms; but when ammonium nitrate was included, a rich growth followed. This means that some substance present in the agar enabled the fungi to utilize ammonium nitrate. While the Lassaigne test showed only a faint trace of nitrogen in agar-agar, we feel safe in assuming that this small amount of organic nitrogen was sufficient to give the mycelium the necessary start in the utilization of ammonium nitrate.

Since agar-agar merely duplicates, to a slight degree, the effect of the amino acids in the medium, the interpretation of data herein tabulated should not be complicated by the presence of agar.

THE EFFECT OF AMINO ACIDS AND THIAMIN

Some 24 individual amino acids were used singly and in several combinations. It was found that only 2 organisms, *Basidiobolus ranarum,* as shown before, and *Saprolegnia parasitica,* made good growth without the presence of thiamin. *Basidiobolus ranarum* made excellent growth in the presence of 0.1 per cent *d*-arginine and fair growth in the presence of any one of the following: *l*-aspartic acid, *d*-glutamic acid, glycine, *dl*-*a*-alanine, *dl*-phenylalanine, *l*-histidine, *dl*-valine, *dl*-leucine, and *dl*-serine. *Saprolegnia parasitica* grew only in the presence of either *l*-cystine or *dl*-leucine.

The remaining 23 fungi did not make an appreciable response to amino acids alone, although some were slightly stimulated. We are inclined to think that this stimulation was caused not by the amino acids themselves, but by the minute quantities of impurities present in them. A substance may be chemically pure, but biologically impure, because microorganisms are much more sensitive than chemical tests. Different lots of the same amino acid purchased from different manufacturers, and even the age of a given amino acid sold by the same firm, seemed to influence growth-stimulating properties. This is particularly applicable to the "natural" amino acids.

Combinations in 2's, 3's and 4's, as well as a mixture of all 24 of the amino acids, failed to show a marked advantage over the use of a single one in so far as the individual response of any one organism was concerned. However, where several organisms are to be cultured on a common medium, a combination of the more favorable amino acids will prove advantageous because, whereas one fungus attains its maximum growth in the presence of arginine, another does so in the presence of *dl*-*a*-alanine, or aspartic acid, etc. In case of fungi with a limited range of amino acid preference, such as *Saprolegnia parasitica,* the addition of either *l*-cystine or leucine to the mixture of amino acids will effectively extend the general usefulness of the medium.

In view of the response made by *Saprolegnia parasitica* to *l*-cystine, it was believed that some other organisms, which failed to grow on the synthetic

medium with the group of amino acids as source of nitrogen, and failed to respond to thiamin, probably would grow if the medium contained *l*-cystine. This proved to be the case, and *Saprolegnia mixta, Achlya conspicus, Isoachlia monilifera,* and *Aphanomyces camptostylus* made a rich growth.

It might be of interest to note the findings of other investigators concerning the effect of amino acids. Fildes and Richardson (5) have shown that tryptophane, leucine, phenylalanine, tyrosine, and arginine are indispensable for the growth of *Clostridium sporogenes,* while histidine, cystine, methionine, and valine are highly important and possibly indispensable. Anderson and Emmart (1) found that glycine, *l*-leucine, *l*-tyrosine, and *l*-aspartic acid did not produce stimulation of metabolism in *Fusarium oxysporum,* but *l*-aspartic acid and glycine were utilized by the fungus for the production of carbon dioxide, whereas leucine and tyrosine retarded the production of this gas. According to Tatum, Peterson, and Fred (32) *l*-asparagine is the factor in potato extract that stimulates butyl alcohol formation by certain butyric acid bacteria, and Tatum, Wood, and Peterson (33) have shown that the ammonium nitrogen and the asparagine of Neuberg precipitate fraction of potato extract stimulated a number of propionic acid bacteria. Mosher, Saunders, Kingery, and Williams (12) tested the effect of a number of amino acids upon *Trichophyton interdigitale* and found that no single amino acid was indispensable. Some proved to be very helpful, others less necessary, while still other amino acids had no specific effect. They found a varied assortment of amino acids to be superior to any group of 3 or 4; yet, given an adequate assortment of amino acids, the fungus would not grow unless supplied with at least one of the following: pantothenic acid, inositol, thiamin, or crude lactoflavin. These workers state that leucine proved the most important individual amino acid, with aspartic acid and *B*-hydroxy-alpha-amino butyric acid next in importance. Nielson (15) used 32 different amino acids in his work on nitrogen assimilation by *Saccharomyces cerevisiae* and observed a 99 per cent of assimilation of the following amino acids by the yeast: glycine, *dl*-aspartic acid, *l*-aspartic acid, and asparagine; 92–97 per cent assimilation of nitrogen was observed in case of *d-alanine, l*-tyrosine, *l*-leucine, *d*-*iso*-leucine, *d*-asparagine, and *dl*-glutamic acid. The yeast could not utilize *dl*-diaminopimelic acid, and only 1 per cent assimilation was noted in case of *d*-lysine and *dl*-diaminoadipic acid. All others varied from 2 to 81 per cent. Schopfer (24) states that in the presence of thiamin and with arginine taking the place of asparagine in the nutrient medium, *Phycomyces blakesleeanus* produced excellent growth, while tyrosine, lysine, and ornithine proved ineffective; serine and *dl*-proline were fair in this respect, and *d*-alanine, *l*-leucine, *d*-leucine, glumatic acid, hydroxyproline, and glycyl-glycine varied from slight to poor.

It is a well-known fact that different organisms do not always respond alike to the same food substances. Nor can we expect all fungi to utilze the same amino acids either with or without the aid of accessory growth factors. The table 3 illustrates this point. Twenty-four amino acids of 0.1

per cent concentration were tested separately, each replacing the ammonium nitrate of the synthetic medium. Crystalline thiamin at the rate of 1 part in 20 million was used as the accessory factor. This concentration of the vitamin is an arbitrary one; much greater dilutions gave equally good results and much higher concentrations failed to cause greater acceleration. Noticeable effects were seen, even in such a great dilution as one part of the vitamin in ten billion parts of the medium.

All results shown in table 2 are approximate; the exact relation of each organism to each of the amino acids tested can be determined only by the dry weight of the mycelium grown in nutrient solutions. Such a procedure probably would alter somewhat the order of availability of the amino acids, but the results would hardly justify the enormous amount of work involved.

The table 3 shows that every one of the 14 fungi is capable of making a good growth if given only one favorable amino acid as the source of nitrogen, and crystalline thiamin as the accessory factor. This seems not to accord with the results of some other workers who find that a given test organism requires more than one amino acid for best growth. The question is whether certain organisms utilize the amino acids *per se,* being incapable of synthesizing their own, or whether they must first break down the amino acids into more easily available forms and then proceed to build their own amino acids. If we accept the first theory, we shall be forced to admit that certain fungi can synthesize some of their amino acids, but must secure others from extraneous sources because proteins result from the combination of a number of amino acids instead of a few. As shown in table 1, 10 organisms can grow well in a synthetic medium with ammonium nitrate as the only source of nitrogen and thiamin as the accessory factor. Obviously, all 10 of these fungi are capable of synthesizing their own amino acids from the ammonium nitrate. Four additional organisms made a very good growth when the ammonium nitrate of the medium was substituted with a suitable amino acid. Since one amino acid does not make a protein, and since no organism used in this work has grown better on a combination of amino acids than on a single one, it is concluded that all 14 of the organisms manufacture their own amino acids. Nitrogen metabolism in these fungi depends on 3 factors: a suitable source of nitrogen, thiamin, and the enzymes of the organisms. Presumably the vitamin acts as a catalyzer; being effective in extremely minute quantities, the likelihood of its utilization as food is remote. This catalytic action probably occurs within the protoplasm in the presence of endoenzymes. Unless combined with some of the end products and rendered inactive, the vitamin should be present in the growing mycelium. Schopfer and Jung (30) were unable to obtain a positive test for thiamin when they fed to the experimental animals the mycelium of *Phycomyces blakesleeanus* grown in a medium containing the vitamin. This suggests one of the following possibilities: the vitamin was destroyed or reduced to its simpler components; it was combined with certain metabolic by-products and rendered inactive, or the mycelium did not contain enough thiamin to

TABLE 3.—*Amino acids as source of nitrogen with thiamin as accessory factor*

Organisms	The amino acids that were utilized by the fungi with the aid of thiamin
Blakesleea trispora	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-a*-amino-caproic acid, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *l*-proline, *dl*-leucine, *dl*-serine. Utilized, but not so well: *dl-iso*-leucine, *dl-a*-amino-*n*-caprylic acid, β-alanine, *l*-tyrosine.
Chaetocladium brefeldii	Glycine, *dl*-alanine. Utilized, but not so well: *d*-arginine, *dl-iso*-leucine.
Collybia tuberosa	*d*-arginine, *l*-aspartic acid. Utilized, but not so well: *dl*-serine.
Coprinus lagopus	*l*-aspartic acid, *d*-glutamic acid. Utilized, but not so well: *l*-tryptophane, *dl-iso*-leucine, *dl-a*-amino caprylic acid.
Lentinus tigrinus	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl*-phenyl-alanine, *dl-iso*-leucine, *l*-cystine, *dl*-valine, *dl*-serine. Utilized, but not so well: *l*-tryptophane, *dl-a*-amino-*n*-caproic acid, *dl-a*-amino-caprylic acid, *dl-a*-amino-*n*-butyric acid, *l*-proline, *dl*-leucine.
Nyctalis asterophora	*d*-arginine, *d*-glutamic acid, glycine. Utilized, but not so well: *l*-aspartic acid, *dl-a*-alanine, *l*-tyrosine, *dl*-serine.
Phycomyces nitens	*d*-arginine, glycine, *dl-a*-alanine. Utilized, but not so well: *l*-aspartic acid, *d*-glutamic acid, *dl-iso*-leucine, *dl-a*-amino-caprylic acid, *l*-histidine, *dl*-serine.
Pilaira moreaui	Glycine, *dl*-alanine, *dl-iso*-leucine. Utilized, but not so well: *d*-arginine, *l*-aspartic acid, *d*-glutamic acid, β-alanine, *dl-a*-amino-caprylic acid, *l*-histidine, *l*-tyrosine, *dl*-leucine, *dl*-serine.
Phytophthora erythroseptica	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *l*-histidine, *l*-proline, *dl*-serine. Utilized, but not so well: *iso*-leucine, *l*-tryptophane, *dl*-valine.
Pleurotus corticatus	*l*-aspartic acid, *d*-glutamic acid. Utilized, but not so well: *d*-arginine, *dl-a*-alanine, *dl-iso*-leucine.
Pythiomorpha gonapodioides	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-iso*-leucine, β-alanine, *l*-histidine, *l*-hydroxyproline, *l*-proline, *l*-tyrosine, *dl*-serine. Utilized, but not so well: *l*-tryptophane, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *dl*-leucine.
Pythiomorpha oryzae	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *l*-histidine, *l*-proline. Utilized, but not so well: *dl-a*-alanine, *l*-tryptophane, *l*-hydroxyproline, *dl*-valine, *l*-tyrosine, *dl*-serine.
Pythium oligandrum	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, glycine, *dl-a*-alanine, *dl-iso*-leucine, *l*-proline. Utilized, but not so well: β-alanine, *dl-a*-amino-*n*-butyric acid, *dl*-valine, *dl*-serine.
Pythium polymastum	*d*-arginine, *l*-aspartic acid, *d*-glutamic acid, *dl-a*-alanine, β-alamine, *l*-proline, *dl*-serine. Utilized, but not so well: glycine, *l*-histidine, *l*-hydroxyproline, *dl*-leucine.

supply the needs of the experimental animals. As fungi respond to much more minute doses of thiamin than do animals, an optimum dose for a fungus may still fall below the minimum for animals.

We used the following fungi to demonstrate whether or not a given organism is capable of manufacturing thiamin:

Cunninghamella blakesleeana grows very well on synthetic media containing neither thiamin nor constituents of thiamin. It was grown in 2 series of solutions, the first consisting of our synthetic medium with ammonium nitrate as the only source of nitrogen, and the second, containing our mixture of amino acids as the source of nitrogen.

Pythiomorpha gonapodioides was grown in the synthetic medium with the amino acids as source of nitrogen plus a mixture of the 2 intermediates of thiamin (2-methyl-5-ethoxymethyl-6-amino pyrimidine and 4-methyl-5-β-hydroxyethyl thiazole). Another series consisted of the same solution but containing only pyrimidine.

Phycomyces nitens was grown in the nutrient solution containing pyrimidine and thiazole.

Blakesleea trispora was grown in the synthetic solution containing amino acids and pyrimidine. The concentration of pyrimidine and thiazole was the same in all cases, 0.05 ppm. Each flask contained 200 ml. of the medium.

After a growth of 2 weeks in diffused light and at room temperature, the mycelium was removed, thoroughly washed, ground, added to the nutrient solution with the amino acids as source of nitrogen, heated in the autoclave for 5 minutes at 5 pounds' pressure, filtered, sterilized, and inoculated with: 1. *Pythiomorpha gonapodioides,* which grows readily in the synthetic solution containing pyrimidine only. 2. *Pilaira moreaui,* which requires both constituents of thiamin. 3. *Phycomyces nitens,* which has the same requirements as *Pilaira moreaui*. 4. *Phytophthora erythroseptica,* which cannot grow unless thiamin is present.

All 4 organisms grew very well in the presence of the mycelium extract of the 4 fungi listed above. Since *Phytophthora erythroseptica* grew very readily in all cases, we can safely assume that *Cunninghamella blakesleeana* synthesized its own thiamin from the synthetic solution; that *Pythiomorpha gonapodioides* can synthesize thiamin when furnished with pyrimidine, or from a mixture of pyrimidine and thiazole; that *Phycomyces nitens* prepares its own thiamin from a mixture of pyrimidine and thiazole; and that *Blakesleea trispora* can do the same with only pyrimidine in the solution. Some of the thiamin manufactured by the fungus is given off by the mycelium into the medium. In case of *Blakesleea trispora* the nutrient solution from which the mycelium was filtered off was tested and proved active for *Phytophthora erythroseptica*. Since the latter organism cannot grow in the presence of pyrimidine alone, it is a safe assumption that *Blakesleea trispora* excreted thiamin into the medium. The mycelium extract was more active than the medium.

Robbins and Kavanagh (31) have evidence showing that an organism

that grows well with an external supply of pyrimidine alone is capable of synthesizing thiazole, although it does not grow with thiazole alone. We have demonstrated that fungi synthesize their own thiamin from purely synthetic solutions, or from only one of the constituents of the thiamin, or from a mixture of pyrimidine and thiazole. If either constituent were capable of doing the work of thiamin, then why the elaborate process of thiamin synthesis? We agree with Robbins and Kavanagh that thiamin itself is the active agent in the metabolic activities of fungi and not its individual components.

THE EFFECT OF THE TWO INTERMEDIATES OF THIAMIN

Vitamin B_1 is a complex compound formed synthetically from 4-methyl-5-β-hydroxyethyl thiazole and 2-methyl-5-bromomethyl-6-amino pyrimidine. The question that naturally presents itself is whether these 2 intermediates are capable of replacing thiamin in nutrition tests before being linked. Schopfer (28) finds that the pyrimidine which he used (2-methyl-4(6)-amino-5-aminomethyl pyrimidine) increased the growth of 2 species of *Rhodotorula*, *R. rubra* and *R. flava*, whereas the thiazole was without any effect on *R. rubra* and slightly favorable to *R. flava*. Schultz, Atkin, and Frey (31) observed that Type A yeast was partly stimulated by either thiazole or pyrimidine and completely activated by the mixture of the two. Type B, on the other hand, was unaffected by thiazole, slightly inhibited by pyrimidine, and greatly inhibited by the mixture of the two. Schopfer and Jung[3] state that *Phycomyces blakesleeanus* is unable to grow in the presence of either thiazole or pyrimidine alone, but when these two are mixed, a good growth follows. Robbins and Kavanagh (18) obtained similar results with this organism, and Robbins (22) extended the work to numerous other organisms. Robbins and Kavanagh (21) confirmed Schopfer's results and extended their investigations to include a number of additional fungi. They demonstrated that, given the pyrimidine moiety, some were able to synthesize their own thiazole. Schopfer (29) states that *Absidia ramosa* and *Parasitella simplex* make only a slight growth in the control medium, or in the control to which thiazole is added, whereas in the presence of pyrimidine alone a fairly good growth follows. The maximum crop of mycelium, however, cannot develop without the presence of both intermediates or of thiamin. Müller and Schopfer (14) were unable to grow *Mucor ramannianus* without thiamin; pyrimidine failed to replace thiamin, but thiazole proved as effective as thiamin.

Through the courtesy of Merck & Co. we obtained the 2 intermediates of thiamin and used them at the same concentration as the vitamin (0.05 ppm.) with the amino acid mixture as the source of nitrogen. Table 4 gives the results.

When used alone, thiazole was unable to induce growth in any of the fungi, but, mixed with pyrimidine, it became a vital factor in the growth of *Chaetocladium brefeldii, Lentinus tigrinus, Pilaira moreaui,* and *Phycomyces*

[3] *Loc. cit.*

TABLE 4.—*The growth promoting qualities of the two intermediates of thiamin*

4-methyl-5-β-hydroxyethyl thiazole, 0.05 ppm. in nutrient medium	2-methyl-5-ethoxymethyl-6-amino pyrimidine, 0.05 ppm. in nutrient medium	0.05 ppm. each of the thiazole and pyrimidine in nutrient medium
No growth by any of the organisms	Good growth *Blakesleea trispora* *Collybia tuberosa* *Coprinus lagopus* *Nyctalis asterophora* *Pleurotus corticatus* *Pythiomorpha gonapodioides* *Pythium oligandrum* Poor to fair growth *Lentinus tigrinus* *Pilaira moreaui* No growth *Chaetocladium brefeldii* *Phycomyces nitens* *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*	Good growth *Blakesleea trispora* *Chaetocladium brefeldii* *Collybia tuberosa* *Coprinus lagopus* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pleurotus corticatus* *Pythium oligandrum* No growth *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*

nitens. Only 3 organisms (*Phytophthora erythroseptica, Pythiomorpha oryzae,* and *Pythium polymastum*) remained unaffected by the pyrimidine, or by the pyrimidine and the thiazole mixed. These 3 fungi must have thiamin, which, apparently, is not broken up by the organisms into its 2 constituents before being utilized.

OTHER THIAZOLES AND PYRIMIDINES AS ACCESSORY FACTORS

After the synthesis of thiamin was accomplished, many analogues of vitamin B_1 were prepared and tested on experimental animals. Unfortunately, these are not generally available, and we know of only Robbins and Kavanagh (20) who have tested at least one of these analogues on fungi. Their experimental organism, *Phycomyces blakesleeanus,* failed to make any growth on it; upon the addition to the analogue of the pyrimidine fraction of vitamin B_1, a good growth followed. This probably was because the modification in the analogue was in the pyrimidine moiety, the thiazole remaining the same. In addition to the thiamin analogues, there have been many pyrimidines and thiazoles prepared and tested. Robbins and Kavanagh (20) have done the most extensive work along this line, using *Phycomyces blakesleeanus* as test organism. They tried some 37 various pyrimidines in conjunction with 4-methyl-5-β-hydroxyethyl thiazole and found that the pyrimidine compounds were active only when they possessed an amino group in position 6 and a mono-substituted methyl group in position 5. They (19) also tested 13 various thiazoles in conjunction with 2-methyl-5-bromomethyl-6-aminopyrimidine and 2-methyl-5-ethoxymethyl-6-amino pyrimidine, and found that activity for thiazoles depends upon their configuration; there must be a hydrogen in position 2 and a β-hydroxyethyl group in position 5; the hydroxyl must be free for greatest activity.

We tested 5 different pyrimidines; the test organisms were those of our fungi that responded to the pyrimidine nucleus of thiamin, or to pyrimidine and thiazole mixed. We confirm the conclusions of Robbins and Kavanagh[4] that the amino group in position 6 is necessary for activity. At a concentration of 0.05 ppm. we found no difference in activity with the following groups in position 5: ethoxymethyl, hydroxymethyl, and bromomethyl. When the amino group in position 6 was replaced by chlorine or oxygen, the activity was lost. We tested 2-methyl-5-hydroxymethyl-6-amino pyrimidine and found it to be equally effective as 2-methyl-5-bromomethyl-6-amino pyrimidine or 2-methyl-5-ethoxymethyl-6-amino pyrimidine. Thus the $-CH_2OH$ group may be added to the list of Robbins and Kavanagh ($-CH_2Br$, $-CH_2OC_2H_5$ and $-CH_2NH_2$) as being an effective substituant in position 5.

We also tested 4 different thiazoles and observed that ethyl-4-methyl-thiazole-5-carboxylate (H. Clarke) was able to replace 4-methyl-5-β-hydroxyethyl thiazole for some of the organisms that require both constituents of thiamin. We confirm Robbins and Kavanaugh that 4-methyl-5-β-ethoxyethyl thiazole picrate (H. Clarke) is less active for *Phycomyces blakesleeanus* than 4-methyl-5-β-hydroxyethyl thiazole, although no such decrease in activity was noted in case of the other organisms.

THE EFFECT OF PROLONGED AUTOCLAVING UPON THE ACCESSORY GROWTH FACTORS

It is a well-established fact that thiamin in alkaline solution (pH 10) is destroyed when autoclaved at 15 lb. for 5 hours. The nature of this destruction is not known; the vitamin may break down into simpler compounds or perhaps a molecular rearrangement takes place and destroys the prophylactic property of the vitamin. Since thiamin apparently does not always function the same way for fungi as it does for animals, it would be of interest to find what effect, if any, prolonged autoclaving of the vitamin would have upon the growth response of fungi.

Four solutions were prepared: the first contained yeast extract (1000 ppm.), to the second was added thiamin (0.05 ppm.), the third had pyrimidine, and the fourth thiazole and pyrimidine (0.05 ppm. each). The solutions were adjusted to pH 10 by means of sodium hydroxide and autoclaved continuously for 5 hours at 15 lb. The pH was then adjusted to 5.5 with dilute sulphuric acid, the nutrient constituents and agar were added, tubed, sterilized, and inoculated. Table 5 summarizes the results:

Only 4 out of 14 fungi failed to grow in the medium containing the autoclaved thiamin, the remaining organisms, apparently, are capable of utilizing the by-products that result from thiamin decomposition. Four organisms (4th column) failed to grow in the presence of the autoclaved pyrimidine and thiazole mixture, although in this instance failure to grow cannot be ascribed to autoclaving, as, with the exception of *Collybia tuberosa*, the other 3, *Pythium erythroseptica*, *Pythiomorpha orzae*, and *Pythium polymastum*, do not respond to a mixture of pyrimidine and thiazole. In case of *Phycomyces*

[4] *Loc. cit.*

TABLE 5.—*The effect of prolonged autoclaving upon the growth promoting properties of yeast, thiamin, and the two components of thiamin (5 hrs. 15 lbs. pH 10)*

Yeast extract 1000 ppm.	Thiamin 0.05 ppm.	2-methyl-5-ethoxy-metyl-6-amino pyrimidine, 0.05 ppm.	2-methyl-4-ethoxy-methyl-6-amino pyrimidine 0.05 ppm. plus 4-methyl-5-hydroxyethyl thiazole 0.05 ppm.
Good growth *Blakesleea trispora* *Chaetocladium brefeldii* *Coprinus lagopus* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pleurotus corticatus* *Pythium oligandrum*	Good growth *Blakesleea trispora* *Coprinus lagopus* *Lentinus tigrinus* *Nyctalis asterophora* *Pythiomorpha gonapodioides* *Pleurotus corticatus* *Pythium oligandrum*	Good growth *Blakesleea trispora* *Coprinus lagopus* *Nyctalis asterophora* *Pythiomorpha gonapodioides* *Pythium oligandrum*	Good growth *Blakesleea trispora* *Coprinus lagopus* *Lentinus tigrinus* *Nyctalis asterophora* *Phycomyces nitens* *Pilaira moreaui* *Pythiomorpha gonapodioides* *Pleurotus corticatus* *Pythium oligandrum*
Fair growth *Pythium polymastum*	Fair growth *Chaetocladium brefeldii* *Pilaira moreaui* *Pythium polymastum*	Fair growth *Lentinus tigrinus* *Pleurotus corticatus*	Fair growth *Chaetocladium brefeldii*
No growth *Collybia tuberosa* *Phytophthora erythroseptica* *Pythiomorpha oryzae*	No growth *Phycomyces nitens* *Collybia tuberosa* *Phytophthora erythroseptica* *Pythiomorpha oryzae*	No growth *Chaetocladium brefeldii* *Collybia tuberosa* *Pilaira moreaui* *Phycomyces nitens* *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*	No growth *Collybia tuberosa* *Phytophthora erythroseptica* *Pythiomorpha oryzae* *Pythium polymastum*

nitens the autoclaved mixture of pyrimidine and thiazole remained active, although autoclaved thiamin lost all activity. *Pythium polymastum,* which in previous experiments seemed to require thiamin, made a fair growth in the presence of autoclaved thiamin, as well as of yeast extract. This suggests two possibilities: either this organism is capable of utilizing some thiamin by-product or else it is able to dissociate into a form capable of using some of the intermediates. Dissociation is by no means uncommon, and 2 organisms in particular, *Chaetocladium brefeldii* and *Lentinus tigrinus,* have given some erratic responses that cannot be explained by any other theory.

FRACTIONATION OF YEAST EXTRACT

It has been seen that yeast extract is capable of supporting growth not only in organisms requiring thiamin or its moieties but also in those fungi that do not respond to thiamin. We endeavored to separate these 2 factors from the yeast extract. It should be stressed here that chemical methods of separation and concentration may often be very destructive. For instance, from 250 kg. of dried egg yolk, Kögl obtained only 1.1 mg. of biotin. He

estimates that over 98 per cent of the biotin was destroyed during the process of purification. Perhaps this is one reason why animal physiologists have purified so few of their "factors" found in yeast.

The yeast auxithals that induced growth in our test organisms are readily adsorbed on neutral norit from which they may be eluted by means of hot pyridine-water (2 : 1). The non-adsorbed fraction, consisting of 90 per cent of the original yeast extract proved entirely inactive. The eluate was dried in vacuo (20 mm.), dissolved in pyridine-water and precipitated by 10 volumes of 95 per cent ethanol. The precipitate, being inactive, was discarded. The filtrate was taken to dryness, dissolved in 90 per cent formic acid, and precipitated by 15 volumes of acetone. The inactive precipitate was discarded; the filtrate was taken to dryness by vacuum distillation, dissolved in 5 per cent sulphuric acid, and precipitated with 50 per cent phosphotungstic acid in 5 per cent sulphuric acid. The filtrate was inactive. The precipitate was regenerated with barium hydroxide and sulphuric acid and, after drying, extracted with hot absolute alcohol. The residue was discarded, the filtrate was "esterified" by adding 1 ml. of concentrated sulphuric acid and by refluxing for 2 hours. The sulphuric acid was removed with barium carbonate and the alcohol by distillation. The dry residue was then treated with boiling chloroform. The chloroform-soluble fraction (A) proved active for organisms requiring thiamin, while the chloroform-insoluble fraction (B) proved active for the organisms that failed to respond to thiamin.

Vacuum sublimation at 0.1 mm. and 150° C. of the material regenerated from the phosphotungstic precipitate yielded needle-like, double-refractive crystals that proved active for the organisms requiring thiamin, but inactive for the other group. The unsublimed residue proved active for organisms that failed to respond to thiamin. A more clear-cut separation was obtained by sublimation than by chloroform fractionation. There was, however, a greater destruction of fraction B because of the high temperature used in sublimation.

Fraction A may or may not be a mixture of thiazole and pyrimidine, but we feel safe in assuming that the needle-like crystals we have separated cannot be a mixture of these two. The greatest dilution in which these crystals show noticeable activity is 1 part in 5 million, whereas pyrimidine, or pyrimidine and thiazole in mixture, is highly active in much greater dilutions. It is possible that the crystals are inactive and that they carry the active principle as adsorbed impurity. Further work will clear this point.

Fraction B is bios-like in its action; it supports excellent growth of various strains of *Saccharomyces cerevisiae*. Possibly, it is related to biotin or contains biotin in addition to other auxithals. It has, however, about the same degree of activity as fraction A, which shows that its biotin content is rather small. (Pure biotin is active in 1 part per 250 billion.) We have fairly good evidence that this fraction contains biotin because *Ashbya gossypii* does not make any growth in the presence of fraction B in our nutrient medium nor in the presence of inositol, but, when inositol (100 ppm.) and fraction B (10

ppm.) were added together to the medium, a rich golden growth resulted. Kögl and Fries (6) obtained similar results with this organism when they used biotin and inositol, whereas no growth occurred when these two were tested separately.

SUMMARY

Twenty-five fungi used as test organisms failed to grow on a synthetic medium consisting of 1 g. ammonium nitrate, 0.5 g. each of dihydrogen potassium phosphate and magnesium sulphate, 5 g. of dextrose, 20 g. of agar, and 1,000 ml. of distilled water. When a minute quantity of crystalline thiamin was added to this medium (0.05 ppm.) ten of the fungi grew very well; but when a mixture of amino acids was substituted for ammonium nitrate, 4 additional organisms made a good growth.

Sodium nitrate, sodium nitrite, potassium cyanide, and urea were substituted for ammonium nitrate in the foregoing medium, with the vitamin as the accessory factor. Only 1 organism made some growth in the medium containing sodium nitrite. Sodium nitrate, potassium cyanide, and urea were not so favorable as ammonium nitrate.

Twenty-four amino acids were used singly or in combinations to replace the ammonium nitrate of the medium. In case of 2 organisms, *Basidiobolus ranarum* and *Saprolegnia parasitica,* amino acids proved to be the growth-inducing substances; the remaining 23 were unaffected. The addition of thiamin to the proper amino acids induced growth in 14 fungi. Nine organisms failed to respond to the amino acids plus thiamin.

The 2 intermediates of thiamin were tested, 4-methyl-5-β-hydroxyethyl thiazole was inactive when used alone; 2-methyl-5-ethoxymethyl-6-amino pyrimidine induced growth in 9 organisms; the 2 constituents together induced growth in 11 organisms; 3 fungi responded only to thiamin.

Five different pyrimidines and 4 different thiazoles were tested, confirming that the amino group in position 6 is necessary for the activity of pyrimidine and that there must be a hydrogen in position 2 and a β-hydroxyethyl group in position 5 for the activity of thiazole.

Solutions of thiamin, of pyrimidine, and of pyrimidine and thiazole together were adjusted to pH 10 and autoclaved for 5 hours at 15 lb., adjusted to pH 5.5, added to the nutrients and tested. Autoclaved thiamin induced growth in 10 organisms as against 14 that grew in media containing non-autoclaved thiamin. Some organisms that failed to grow in the presence of autoclaved thiamin grew when the autoclaved pyrimidine and thiazole mixture was added to the medium.

A method is described whereby yeast extract was fractionated to yield two components. The first of these induced growth in the organisms that responded to a mixture of thiazole and pyrimidine, while the other fraction proved active for the organisms that failed to respond to thiamin or its intermediates.

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ACQUIRED TOLERANCE TO CURLY TOP IN THE TOMATO[1]

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When young tomato plants under field conditions are inoculated in late spring with the curly-top virus, symptoms of the disease usually appear on infected plants in about 12 days. The leaves become rolled and the plant assumes a sulphur-yellow color. Growth is checked, flowers and buds drop, and the fruit colors prematurely. Plants that have already matured fruit, and those raised from cuttings of more mature plants are less easily infected, but when infection occurs the symptoms are the same. As a rule, the affected plant dies, but in some instances severely diseased plants have been observed to make new growth, which suggests at least a partial recovery from the disease.

The term "recovery" in the present sense means a process of regeneration in which relatively healthy shoots arise from the leaf axils. New, apparently healthy shoots sometimes arise from the lower nodes of severely diseased plants after the almost complete death of the top-growth. If a plant recovers sufficiently early in the season, and if the growth of the regenerated parts is more or less normal, the plant may yield mature fruit. The new growth is usually not quite normal, especially at the beginning. It may be feeble and yellowish, or fairly healthy, with only a slight yellow and purple tinge suggesting mild symptoms of curly top.

If recovery be slow and feeble a relapse may occur, resulting in death of the plant. The factors causing the relapse of tomato plants after they show recovery are not understood. A case is reported by Lesley[4] of a recovered

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TABLE 1.—*Observed cases of recovery from curly-top symptoms by regeneration in tomato seedlings inoculated at Riverside, California, 1928–1937*

Races, hybrids, and commercial varieties	Number of plants				Percentage recovered
	Inoculated	Diseased	Recovered	Relapsed	
WILD RACES AND RACE HYBRIDS:					
Lycopersicum pimpinellifolium No. 497	10	10	5	2	50
" " F.P.I. 79532	10	9	5	0	56
" " F.P.I. 97850	20	19	19	0	100
No. 381–1		3[a]	1	0	33
No. 503	26	26	11	0	42
F_1; No. 573 × *L. pimpinellifolium* No. 548	10	10	1	0	10
F_1; No. 503 × " " F.P.I. 79532	24	13	10	0	83
F_1; No. 573 × " " F.P.I. 79532	23	11	0	0	0
F_1; No. 381–1 × " " No. 548	23	10	4	0	40
Guasave A	40	37	33	1	89
Guasave A, Recovered parents selfed	22	22	12	3	55
Guasave B	8	6	2	0	33
Ecuador	11	11	9	5	82
COMMERCIAL VARIETIES:					
Stone, Norton, Santa Clara, Marglobe	211	131	9	4	7

[a] These plants became infected naturally.

tomato plant that, following a heavy inoculation the same season, again developed severe curly-top symptoms. However, in most instances reinoculation of recovered plants has had no visible effect on the plants.

Under field conditions, plants that develop curly-top symptoms early in the season are probably less likely to recover than those developing symptoms later. In 1931 and 1933, at Riverside, among 53 plants that showed curly top early in the season, there was 15 per cent recovery, while of 20 plants that developed symptoms later in the season there was 25 per cent recovery.

Although recovery by regeneration appears to be influenced both by weather conditions and the stage of growth of plants at the time of appearance of symptoms, the ability to recover is a racial characteristic also. Some cases of recovery in tomato races and hybrids observed at Riverside from 1928 to 1937 are listed in table 1. In one wild race, Guasave A, from western Mexico, the proportion of plants that recovered was especially high, varying from 55 to 100 per cent in different years. In other races, including several commercial varieties not listed in table 1, hardly any plants recovered. A race from Ecuador was "high" in recovery but seemed subject to relapse.

A small number of F_1 plants of "high recovery" wild races, No. 503 × F.P.I. 79532 was high in recovery. F_1 hybrids of high-recovery race F.P.I. 79532 and another *pimpinellifolium* race No. 548 with No. 573, a wild race whose power of recovery is not known, were decidedly low in recovery. Seedlings from a recovered plant seem to have no greater ability to recover than seedlings from the ordinary stock seed of the race.

Clones derived from tomato plants that recovered from curly top by regeneration have an apparent resistance or tolerance not present in clones from disease-free, noninoculated plants. The results of inoculations of clones from both sources are compared in table 2.

In 1934, 7 plants from cuttings of hybrid C 263–1, which had recovered the previous year, were exposed to infection in the field by caging 10 viru-

TABLE 2.—*Effect of inoculation of tomato clones derived from plants that recovered from curly top and of clones from healthy, noninoculated plants*

Parentage of clones	Number of progeny plants	
	Inoculated	Diseased[a]
Clones from recovered parents:		
Hybrid C 263–1 (Wild)	7	0
No. 381–1 (Wild race) × *Lycopersicum pimpinellifolium* No. 548	23	4[b]
Guasave A (Wild)	34	0
Clones from healthy, noninoculated parents:		
Goffs (Wild)	8	6[c]
Selection 36.033.15 (Commercial)	19	13[c]

[a] Clones from recovered parents usually showed slight curly-top symptoms when inoculated.

[b] Symptoms on these plants became more pronounced following inoculations.

[c] These plants became severely diseased and all died.

liferous beet leaf hoppers on each plant. None of these plants developed curly-top symptoms any more pronounced than those shown at the time of reinoculation, whereas 43 per cent of the seedling plants of several races similarly treated developed severe symptoms.

An F_1 plant from 2 primitive races, No. 381–1 and No. 548, which showed recovery in 1933, was propagated by cuttings for 3 years. In 1934, 5 plants, and in 1935, 9 plants of this clone were inoculated in the field. In 1936, 9 plants were inoculated twice during the season. Four of the above 23 plants, at some time following the inoculations, developed more pronounced curly-top symptoms than they had shown at the time of inoculation, but it could not be determined if the increased severity resulted from the inoculations. Two of the 4 plants again recovered and produced a fair crop. The other 19 plants were not visibly affected by the inoculations. During 1935 and 1937, 34 plants from cuttings of recovered plants of Guasave A were reinoculated without any noticeable effect. On the other hand, 27 cuttings from healthy, previously noninoculated plants of 2 other races were inoculated and 19 developed extremely severe symptoms with no recovery.

In order to test for the presence of virus, nonviruliferous beet leaf hoppers were caged on portions of recovered tomato plants for 2 to 3 days and then transferred to healthy seedling beets of a variety susceptible to curly top. Tests were made on 21 tomato plants originating from cuttings from seedlings that recovered the previous year and virus was obtained from each. The percentages of leaf hoppers acquiring virus from the recovered tomato plants and the severity of symptoms produced on the test beets indicated that the tomato plants contained a rather high concentration of virus, and further, that its virulence was unchanged. However, further studies are necessary to determine definitely if any qualitative change of the virus occurs. Tests also were made of plants in the process of recovery. Virus was easily obtained from regenerated, almost symptomless shoots and from severely diseased portions of the same plants. On the other hand, healthy appearing clones taken from plants not experimentally inoculated and not showing curly-top symptoms during the seedling year were tested the following season, before they were inoculated, and were found not to contain virus. When inoculated, such clonal plants developed severe symptoms of curly top. Thus, it seems that the presence of virus in the regenerated plants is a prerequisite for acquired tolerance; at least, tolerance developed only in plants after they had been infected, and the virus remained in the recovered plants and apparently continued to multiply. Nothing is known of how recovered plants would react to reinoculation if they could be entirely freed of virus.

The phenomenon of recovery of tomato plants from curly top is similar to that of recovery of tobacco plants from the ring-spot disease first reported by Wingard,[6] and later studied extensively by Price.[5] In tomatoes, how-

[5] Price, W. C. Acquired immunity to ring-spot in Nicotiana. Contrib. from Boyce Thompson Inst. 4: 359–403. 1932.

[6] Wingard, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U. S.] 37: 127–154. 1928.

ever, fewer of the plants recover and recovery seems not to be so complete as that reported by both Wingard and Price in their studies of tobacco ring spot.

Tomato plants of a recovered clone are relatively slow-growing and have other mild curly-top symptoms characteristic of regenerated shoots. In spite of this condition they may be fairly productive. In 1937, out of 18 plants from a recovered clone of Guasave A that survived a fairly heavy inoculation in addition to the rather high concentration of virus already present, 10 plants produced a good crop of fruit (Fig. 1) and the remainder

FIG 1. Acquired tolerance in a tomato plant of a Guasave A clone derived from a seedling that recovered from curly top. The mature fruits are crimson and averaged 0.5 oz. in weight. (Photo. Oct. 11, 1937.)

a light crop, which matured about the normal time. All the plants were of less than normal size, even allowing for closeness of planting. Reinoculation of such plants in the summer had no noticeable effect on them. Clones from healthy plants of other races inoculated at the same time developed severe curly-top symptoms and many of them died; the surviving affected plants produced little or no fruit. In 1937, 19 seedlings of Guasave A were inoculated and all of them developed severe curly top. All of these seedling plants showed regeneration, but recovery was too late for the production of a crop that season.

In some areas of the western United States curly top is the limiting factor in tomato production. So far, attempts to select or develop resistant commercial varieties have largely failed. No race of tomato, either wild or cultivated, has been found that is highly resistant to initial infection with

curly-top virus. There is some difference in the reaction of certain races or varieties of tomato to curly top but none have shown sufficient resistance to make them of much practical value. Tomato plants affected with curly top do, however, sometimes recover, in part, at least, and acquire a tolerance to the virus. The tomato plants that acquired tolerance to curly top in these studies, with one possible exception, belonged to wild races. Recovery has been observed in cultivated varieties, but, since an intensive study of this problem was begun, sufficient material has not been available to permit conclusions regarding the association of acquired tolerance with recovery in cultivated varieties.

It may be possible to develop by hybridization a desirable tomato, high in recovery from and tolerant to curly top, and of sufficiently early maturity to enable recovered seedlings to produce a satisfactory crop during the first season's growth. However, if a clone of a larger-fruited variety than Guasave A can be obtained, with similar tolerance to curly top, it would be worth while to overwinter it and propagate it by cuttings for use in areas where curly top seriously limits tomato production.

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INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO

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In 1914, Allard (1) found that the failure of *Nicotiana glutinosa* L. to show chlorosis after inoculation with tobacco-mosaic virus was shared by the hybrid *N. tabacum* L. × *N. glutinosa*. This hybrid has since been found to respond to infection with tobacco-mosaic virus, as *N. glutinosa* does, by the production of necrotic primary lesions (7, p. 992; 9). In these lesions the virus is localized except in young plants where systemic necrosis frequently occurs. Whether the necrosis is localized or systemic, ordinary contaminative contacts between leaves of infected and healthy individuals are ineffective in spreading the disease, in contrast to the high infectivity associated with the systemic chlorosis caused by infection with tobacco-mosaic virus in *N. tabacum*.

In preliminary experiments, designed to introduce into *Nicotiana tabacum* the necrotic type of response to infection characteristic of *N. glutinosa*, attempts were made to obtain seed from the first generation hybrid *N. tabacum* × *N. glutinosa*. Plants of this hybrid were grown continuously in greenhouse and garden, often in considerable numbers, for more than 3 years. During this period the hybrid proved consistently self-sterile and sterile to all tested pollens. Finally, because of the failure of repeated attempts to

cross it with varieties of *N. tabacum,* a fertile amphidiploid derived from this hybrid was obtained through the kindness of Dr. R. E. Clausen of the University of California. This amphidiploid species, *N. digluta* Clausen and Goodspeed, was found to resemble *N. glutinosa* in its response to infection with tobacco-mosaic virus. It was therefore used in further breeding experiments. The purpose of this paper is to report the segregation of disease types in successive generations.

EXPERIMENTS WITH DERIVATIVES OF NICOTIANA DIGLUTA

The species *Nicotiana digluta,* described by Clausen and Goodspeed (2) as a self-fertile amphidiploid originating from the hybrid *N. glutinosa* (n = 12) × *N. tabacum* (n = 24), has been studied intensively in the past, but not from the point of view of disease resistance. Derivatives of the form *N. digluta* × *N. tabacum* and (*N. digluta* × *N. tabacum*) × *N. tabacum* were produced and described by Clausen (3). Since they were not tested by inoculation, however, it is not known whether the necrotic type of response was retained in any individuals of this series of hybrids. In such repeated backcrosses, any characteristic dependent on a gene, or genes, introduced from the non-recurrent parent naturally would be eliminated unless specifically demonstrated and retained in each generation.

As a preliminary to the study of disease types in hybrid generations, 75 plants of *Nicotiana digluta* were tested by inoculation with tobacco-mosaic virus, applied by rubbing. They all produced necrotic local lesions resembling those of *N. glutinosa.* On maturing they proved self-fertile and also set seed readily when emasculated flowers were treated with pollen from *N. tabacum.*

In the first hybrid generation, *Nicotiana digluta* × *N. tabacum* var. Connecticut Broadleaf, 132 plants were grown and tested. They all responded to infection with tobacco-mosaic virus by production of necrotic primary lesions like those of *N. digluta* and *N. glutinosa.*

The F_1 plants were crossed with 3 varieties of *Nicotiana tabacum,* Connecticut Broadleaf, White Burley, and Samsoun tobacco. Segregation of the parental disease types occurred among the progeny. Thus (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. Connecticut Broadleaf gave a ratio of 121 necrotic-type plants to 504 chlorotic-type plants. (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. White Burley gave 272 necrotic-type to 269 chlorotic-type plants. (*N. digluta* × *N. tabacum* var. Connecticut Broadleaf) × *N. tabacum* var. Samsoun gave 310 necrotic-type to 357 chlorotic-type plants.

The results of tests of the first backcross generation, together with those of subsequent backcrossed and selfed generations, are shown in table 1.

Except among the derivatives of Connecticut Broadleaf tobacco, it will be seen from the table that in successive generations the ratios of necrotic-type to chlorotic-type plants, though at times deficient, tended to become the typical monohybrid 1 : 1 backcross and 3 : 1 selfed ratios indicative of the

TABLE 1.—*Ratios of necrotic-type to chlorotic-type plants among derivatives of Nicotiana digluta Clausen and Goodspeed*

F_1 hybrid and backcross generations[a]	Number of times with *N. tabacum* as parent[c]	Connecticut Broadleaf backcross line	Burley (Burley 16) backcross line	Samsoun backcross line
N. digluta × *N. tabacum* F_1 ...	2	132: 0	.. ..	.. ..
B_1	3	121: 504	272: 269	310: 357
B_2	4	17: 247	99: 148	93: 171
B_3	5	23: 286	123: 120	96: 149
B_4	6	63: 185	156: 115	120: 135
Selfed generations[b]	**Number of times selfed**	**Connecticut Broadleaf selfed line**	**Burley selfed line**	**Samsoun selfed line**
F_2	1	310: 144	325: 136	324: 110
F_3	2	271: 182	328: 121	399: 0
F_4	3	123: 79	347: 128	303: 0

[a] Beginning with the necrotic-type hybrid *N. digluta* × *N. tabacum* variety Connecticut Broadleaf, backcrosses were made to the three varieties of *N. tabacum*, Connecticut Broadleaf, Burley, and Samsoun, a necrotic-type hybrid of the preceding generation being used as ♀ parent, *N. tabacum* as ♂ parent in each case.

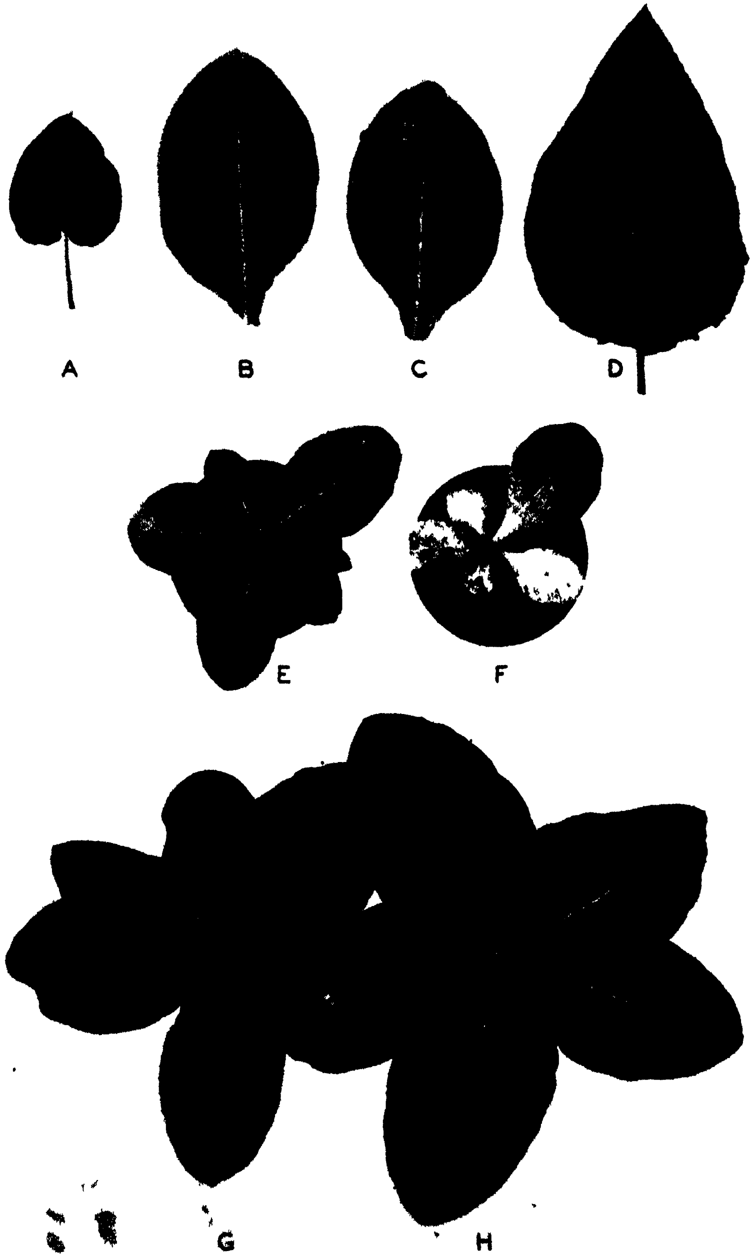
[b] Beginning with a necrotic-type plant of B_1 as origin in each series, a series of filial generations was produced, using a necrotic-type plant of the preceding generation as parent in each case.

[c] Because *N. digluta* itself originally had *N. tabacum* as one parent, and carried a full set of *N. tabacum* chromosomes, the numbers in this column are larger by one than is usually the case for hybrids of the indicated generations.

introduction from *Nicotiana glutinosa* of a single dominant gene inducing necrotic-type response to infection by tobacco-mosaic virus. This gene will be referred to as *N* (necrotic-type response to infection with tobacco-mosaic virus), and its recessive allele, characteristic of *N. tabacum* and of chlorotic-type derivatives of the *N. digluta-tabacum* cross, will be referred to as *n* (chlorotic-type response). Only 2 disease types were observed in the entire series of generations; these were the necrotic and chlorotic types, characteristic of *N. glutinosa* and *N. tabacum*, respectively. No difference was observed between the response of heterozygous (*Nn*) plants and that of homozygous (*NN*) plants. No modification of the action of the *N. glutinosa* gene *N* was detected at any time, despite the varied genetic constitutions of the hybrids into which it was introduced.

In the Burley backcross line, segregation of a pair of genes, presumably the genes G_1g_1 or G_2g_2 of Henika (5), controlling green *vs.* white leaf color, was found to be independent of that of the pair of genes *Nn* controlling necrotic *vs.* chlorotic type of disease. The observed ratios were 71 green necrotic : 69 white necrotic : 70 green chlorotic : 70 white chlorotic, among progeny from doubly heterozygous green necrotic ♀ and doubly recessive white chlorotic ♂ parents.

Throughout this investigation it was found that spread of virus occurred about as promptly in the very youngest necrotic-type plants as in comparable chlorotic-type plants. When early diagnosis of disease type was desired and plants were also to be saved for production of seed, the severity of the sys-



Photographs by J. A. Carlile.

FIG. 1. Leaves 5 days after inoculation with distorting-strain tobacco-mosaic virus, and plants 14 days after similar inoculation. A. From *NN* plant of *Nicotiana glutinosa*. B. From *nn* plant of *N. tabacum*, Burley backcross. C. From *Nn* plant of same. D. From *NN* plant of the repeatedly selfed Samsoun tobacco line; note necrotic lesions in all leaves of plants possessing the dominant gene *N*. E. Young *nn* Burley backcross plant showing *systemic chlorosis*. F. Similar young *Nn* plant showing *systemic necrosis*; contrast with E. G. Older *nn* plant showing systemic chlorosis. H. Comparable *Nn* plant showing only *local necrosis* at site of inoculation. E–G. Inoculated leaf at left.

temic necrosis proved troublesome. It was found, however, that, as plants became slightly older, there was an increasing delay of systemic infection in the presence of the gene for necrosis. It was then possible, after inoculating, to allow systemic chlorosis to appear in all *nn* plants before safeguarding necrotic-type plants by cutting away the inoculated leaves to prevent subsequent escape of virus into stems and top leaves. In still older plants inoculated leaves could be left attached indefinitely without escape of virus to other parts.

Observed deficiencies of necrotic-type plants in progenies derived from Connecticut Broadleaf tobacco, as shown in the third column of table 1, are not entirely understood. Successive generations have shown no indication of any tendency toward establishment of the gene *N*. No simple 1 : 1 backcross or 3 : 1 selfed ratios have occurred thus far. It seems probable, however, that a subsequent approach from a different angle, by hybridization of the necrotic-type Burley and Samsoun derivatives with Connecticut Broadleaf tobacco, may permit the necrotic-type gene to be incorporated in this third variety also.

In figure 1, leaves and plants of necrotic and chlorotic types are represented as they appeared at certain intervals after inoculation. The tendency to virus localization in necrotic-type plants is illustrated.

HOMOZYGOUS NECROTIC-TYPE LINES OF NICOTIANA TABACUM

After the first backcross generation there was little visible indication of any characteristic of *Nicotiana glutinosa* in the hybrids. In successive generations of crosses of necrotic-type plants with 3 varieties of *N. tabacum*, the distinctive characteristics of the 3 horticultural types were soon acquired.

For the purpose of obtaining plants homozygous with respect to the gene *N*, selfed lines were instituted with necrotic-type plants of the first backcross generation as origin. No homozygous sets of plants were attained in the Connecticut Broadleaf selfed line, as might be expected from the failure of this line to produce normal monohybrid ratios. The selfed line of Burley yielded satisfactory 3 : 1 ratios, but this also has not yet given any progenies lacking chlorotic-type individuals. In the selfed line of Samsoun tobacco, however, sets consisting only of necrotic-type plants were obtained. These obviously were derived from homozygous parent plants, in gametes of which the chromosome bearing the gene *N* had been included regularly enough to avoid formation of homozygous recessive-type plants in the tested sample of progeny. Whether complete gametic purity with respect to the gene *N* had been attained was an important question. A more sensitive test of this was given by reciprocal backcrosses to a chlorotic-type tobacco plant

The apparently homozygous necrotic-type (*NN*) Samsoun tobacco derivative, which produced 339 necrotic-type : 0 chlorotic-type plants in the F_3 generation (Table 1), was crossed reciprocally with a plant of the original chlorotic-type variety Samsoun (*nn*), using an individual of the F_3 generation first as pollen parent, then as seed parent. Selfed progeny of the Sam-

soun plant were found to be consistently of chlorotic type (250 individuals tested). Selfed progeny of the F_3 plant were those reported in the table as the F_4 generation (303 individuals tested, all found to be of necrotic type). In each of the reciprocal crosses, 250 plants of the progeny were tested by inoculation. Without exception these proved to be necrotic-type plants. This constituted a critical test of 250 male and 250 female gametes, all of which were thus found to carry the newly introduced gene *N*. A single failure among the 500 would have been disclosed by the appearance of a chlorotic-type plant. To have made an equally sensitive test of gametic purity by self-pollination would have required the square of this number, *i.e.*, 250,000 plants, since the detection of 1 failure in 500 gametes would have been possible only if that gamete happened to fertilize, or to be fertilized by, a similarly rare gamete also failing to carry the gene *N*. The demonstration of gametic purity of the homozygous *NN* line of Samsoun tobacco is thus very satisfactory.

It is believed that similar homozygous lines corresponding to locally desirable horticultural varieties of *Nicotiana tabacum* can be produced readily, either by repeated backcrosses of the homozygous stock of Samsoun tobacco here described, with desired types as recurrent parents, or better by similar crosses of necrotic-type, *Nn* plants of the B_4 generation described in this paper (see table 1). These B_4 plants possess the advantage of having been crossed repeatedly to *N. tabacum* varieties, with opportunity for crossing-over to have transformed the chromosome that bears the newly introduced gene *N* into an essentially *N. tabacum* type of chromosome.

DISCUSSION

Type of Resistance Conferred by the Gene *N*

Immunity from infection by tobacco-mosaic virus is unknown among species of the genus *Nicotiana*. There are, however, important differences in type of response to infection within the confines of the genus (6). Some *Nicotiana* species tend to remain symptomless after they are infected; others show systemic chlorosis. Both the symptomless and the chlorotic-type species, once infected, retain the virus throughout their natural span of life, facilitating spread of virus by providing a good source of inoculum. Still other *Nicotiana* species show systemic necrosis or localized necrosis as a result of infection. The necrotic-type species are protected from plant-to-plant spread of all strains of the virus by early death of invaded tissues, with consequent imprisonment of most of the virus. Their response constitutes an effective type of resistance to the disease from a practical viewpoint, in the sense that spread of the disease through the population is greatly impeded. This particular kind of resistance occurs also among other solanaceous plants, as, for example, in some varieties of eggplant, *Solanum melongena* L. Thus, the Black Beauty eggplant dies as a result of systemic necrosis if infected when young, but localizes the virus in necrotic primary lesions if infected when older (6, p. 333). Under field conditions, injury to this

necrotic-type eggplant is unknown, apparently because no strain of the virus becomes established in large amounts within the crop. Valleau (12) has referred to this type of resistance as depending on *sensitivity* with respect to the virus.

In the past there have been no necrotic-type varieties of tobacco, *Nicotiana tabacum*. All varieties except the symptomless Ambalema tobacco and its derivatives (10, 11) have shown the classical mottling type of systemic chlorosis after infection with ordinary tobacco-mosaic virus. Very large amounts of virus develop in the infected chlorotic-type plants. Consequently, these plants serve during the remainder of their life, and later, when dried, as the principal reservoir from which comes the virus for contaminative infection of later crops of tobacco and other susceptible cultivated species. It is to combat this accumulation of virus in the tobacco crop, rather than to protect the individual infected plant, that a necrotic-type response may prove useful if it can be introduced into horticulturally acceptable strains of tobacco. In the pepper, *Capsicum frutescens* L., complete localization of tobacco-mosaic virus occurs at ordinary temperatures in all plants bearing a dominant gene *L* (7, 8). This localization is permanent because the inoculated leaf is lost by abscission soon after the appearance of necrotic lesions at the site of inoculation. The infected individual is adequately protected. This must not be expected in necrotic-type tobacco, for, although all strains of tobacco-mosaic virus elicit the necrotic type of response, leaf abscission does not follow infection. Both the degree of localization of virus that occurs in old plants and the death of young infected plants, however, would be efficacious in preventing the development of large amounts of inoculum in tobacco. The symptomless condition in the variety Ambalema tends to serve the same purpose with less risk to individual infected plants, but is controlled by a more complex genetic system (4), and is believed to be less uniform in its response to different strains of the virus (12, p. 207).

In the course of the present investigation, a dominant gene for necrotic-type response to infection with tobacco-mosaic virus was incorporated in an inbred line of *Nicotiana tabacum*. This made available a necrotic-type strain of tobacco which is fully fertile with the innumerable tobacco varieties now grown.

The protecting gene is inherited as a Mendelian dominant. Its identification by inoculation methods is readily accomplished. It is hoped, therefore, that further work on the incorporation of the gene into locally acceptable strains of tobacco and subsequent trials under field conditions may be left largely to those who are especially interested in the maintenance and improvement of varieties of tobacco.

Earlier Recognized Functions of the Gene *N*

The dominant gene *N* of *Nicotiana glutinosa*, with which the present investigation is concerned, has played an important rôle in the study of tobacco-mosaic virus, in part even before its identity was recognized. Al-

though its effect in preventing the mottling manifestations of classical tobacco-mosaic disease was the first to be noted, by Allard in 1914 (1, p. 14), this function was not the first to be exploited. Its first important contribution to the scientific investigation of tobacco-mosaic disease was in facilitating quantitative measurement of the causative virus by inducing prompt formation of conspicuous primary lesions, which in great number on a given leaf surface indicated high titer of virus, but, in less abundance, indicated lower titer. A similar gene of *N. rustica* L. and a mechanism of unknown genetic nature in *Phaseolus vulgaris* L. also have been utilized for the same purpose. The ease of performing quantitative measurements thus gained has allowed unusually extensive as well as intensive studies of this virus to be carried out in the decade since 1928, the year in which this method of measurement began to replace earlier, but less efficient and less productive, techniques of minimal inoculation of *N. tabacum* and *N. rustica* plants. The second contribution of this dominant gene was in allowing accurate separation of strains of tobacco-mosaic virus. A similar gene of *N. langsdorffii* Weinm. has been used also for this purpose, permitting many newly derived strains to be promptly separated when they arose from strains already in hand. Its third contribution, in conferring a desirable type of disease resistance, though envisaged first and long delayed in accomplishment, may prove no less important through its apparently decisive control of epiphytotic spread of all strains of tobacco-mosaic virus in *N. tabacum*, the present outstanding source of contaminative infection for susceptible hosts of this virus among crop plants.

SUMMARY

A dominant gene *N*, inducing a necrotic type of response to infection with tobacco-mosaic virus, was transferred from *Nicotiana glutinosa*, through the medium of the amphidiploid species *N. digluta*, into strains of the species *N. tabacum*. By repeated backcrosses of necrotic-type hybrids to *N. tabacum*, tobacco-like derivatives of necrotic type were produced. A homozygous line was then attained by repeated selfings. This homozygous line is self-fertile and fertile with other strains of tobacco. The introduced gene is regularly distributed to its gametes, and so to all individuals of its progeny, whether obtained by selfing or hybridization. It is anticipated that it may prove feasible to incorporate this gene in locally acceptable types of tobacco, to prevent spread of virus from plant to plant within the tobacco crop, and so to eliminate the reservoir of virus in tobacco and tobacco products, the usual sources of infection both for succeeding crops of tobacco and for other crops susceptible to tobacco-mosaic disease.

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EFFECT OF SODIUM CITRATE ON RELEASE OF CURLY-TOP VIRUS FROM ALCOHOLIC PRECIPITATE OF PLANT JUICE[1]

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INTRODUCTION

Certain phases of investigations on the chemical nature of resistance to curly top involved steam distillation of plant material in the presence of a small amount of sulphuric acid. *Atriplex semibaccata* Brown, *Chenopodium murale* L., and *Lycopersicon esculentum* Mill. were thus treated. The large volume of distillate that contained the plant acids was concentrated in each case under reduced pressure 1/20 the original weight of material used and these concentrated fractions were adjusted to pH 7.0.

Tests were made to determine whether or not these concentrated extracts would inactivate the curly-top virus. One ml. of juice from diseased beet plants was added to 4 ml. of each extract. These mixtures were allowed to stand at room temperature for a definite period, usually overnight. The amount of virus present in the mixture was then determined by the method worked out by Bennett.[2]

[1] Contribution from the United States Department of Agriculture, Bureau of Plant Industry, Division of Sugar Plant Investigations, Riverside, California.

[2] Juice was expressed from the severely diseased leaves of 5 to 15 beet plants and centrifuged. To 1-ml. aliquots was added an equal volume of 95 per cent ethyl alcohol. The resulting precipitate was thrown down by centrifugation and the supernatant liquid, discarded. The precipitate was washed once with 50 per cent alcohol, dried, and suspended in water and centrifuged. The supernatant liquid was made acceptable food for the leaf hoppers by adding sufficient sucrose to make a 5 per cent solution. The percentage of leaf hoppers that transmitted the virus from such a liquid to seedling sugar beets indicated

The concentrated fraction from *Atriplex semibaccata* completely inactivated the virus immediately after being prepared. After 11 days of storage in a frozen condition, the same extract[3] lost part of its power to inactivate the virus. When tested a third time, 21 days after the first test, it not only had lost its power to inactivate the virus but liberated large quantities of virus. Inoculation tests resulted in 5-fold increase in infections over the controls. Another extract from *A. semibaccata,* prepared in the same manner as described, apparently had no effect on the virus immediately after being prepared, but on standing 16 days caused an 8-fold increase in infections over that of the controls.

The extract prepared from the juice of the tomato plant liberated the virus from diseased-beet juice, causing a 2-fold increase over the controls. When the fraction from *Chenopodium murale* was tested, sufficient virus was released to double the amount of infection over that obtained in the control tests. Bennett (1) found that 4 parts of juice expressed from *Atriplex semibaccata* inactivated the virus in 1 day when mixed with 1 part of juice from diseased beets.

These results suggested that the curly-top virus is adsorbed by proteins or possibly other substances precipitated from beet-leaf juice by alcohol, and that the salts of the plant acids extracted by steam distillation may, under certain conditions, release large quantities of the virus held in the colloidal complex.

As water is all but nondissociated, extraction with water presumably would not change the electrokinetic properties of the colloidal complex containing the virus; but, if an electrolyte were used, the electrokinetic potential would be shifted and it might be so adjusted as to effect dispersal of more virus.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of Low Concentrations of Certain Electrolytes on the Amount of Virus Released from the Protein Complex

Sodium citrate, because of its trivalent anion, which is largely responsible for its marked influence on the electrokinetic potential, was selected as one of the electrolytes with which to extract the virus from the protein complex. Five-tenths per cent solutions of sodium citrate,[4] sodium acetate, and sodium bicarbonate were prepared for comparative study.

Alcoholic precipitates were prepared in each experiment from mixed juice of 10 to 12 severely diseased plants. This avoided the variation in available virus encountered when single plants are used.

Each of the prepared solutions was tested for its ability to liberate the virus from the alcoholic precipitate. After extraction and centrifuging,

the relative amount of virus in the liquid. Controls in each test were similarly treated, except that 4 ml. of water was added to the juice instead of the extract.

[3] A precipitate slowly formed in the extracts during storage in the refrigerator. When samples were removed for the subsequent tests, only the supernatant liquid was used.

[4] When corrected for the slight moisture content of the anhydrous salt, the concentration of sodium citrate in the solution was 0.438 per cent.

sufficient sucrose was added to the supernatant liquid to bring the concentration to 5 per cent. The liquid was then fed to nonviruliferous leaf hoppers and the customary procedure for inoculation followed. For control tests, alcoholic precipitates were extracted with water and treated in the same manner. A different source of virus was used for each of the 3 experiments shown in table 1.

TABLE 1.—*The effect of certain electrolytes on the amount of curly-top virus released from the alcoholic precipitates of juice from diseased sugar-beet leaves*

Experiment number	Electrolyte added to 5 per cent sucrose	Plants inoculated	Plants infected
		Number	*Number*
38	Control	20	10
	Sodium bicarbonate	20	0
	Sodium acetate	20	11
	Sodium citrate	20	18
	Equal mixture of all 3 salts	20	1
40	Control	20	3
	Sodium citrate	20	12
43	Control	20	0
	Sodium citrate	20	3

The results show that certain electrolytes may play an important part in the release of the virus from the alcoholic precipitate. Sodium citrate released sufficient virus to give double the percentage of infection obtained in the controls. Sodium acetate had no effect on the amount of virus released, while sodium bicarbonate had a depressing effect.

To determine what concentration of sodium citrate would liberate the maximum amount of virus, one liter of a 1.6 per cent solution (54.4 milli-

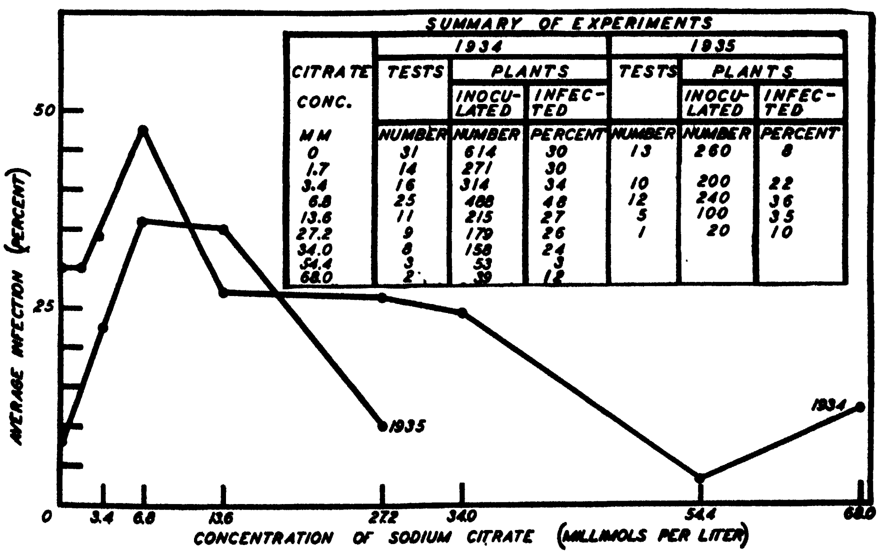


FIG. 1. Release of curly-top virus from alcoholic precipitate of beet-leaf juice with various concentrations of sodium citrate.

molar when corrected for the slight moisture content) of sodium citrate was prepared. Lower concentrations were prepared by diluting in turn 500 ml. of the next higher concentration with an equal volume of distilled water. The results of tests made over a period of 2 years with a range of concentrations of sodium citrate are presented in figure 1.

The percentage of infection reached an abrupt maximum. In the 1934 experiments, 6.8 mM sodium citrate was responsible for a 62 per cent increase in infection over the control tests.

Maximum percentage of infection was not at 6.8 millimolar concentration in all experiments. In 7 experiments in which 3.4 and 6.8 mM concentrations were used on the same source of virus, maximum infection was obtained with the lower concentration. In these 7 tests, the average percentage infection obtained with 3.4 mM was only 10 per cent more than the average values obtained with 6.8 mM sodium citrate.

These data were subjected to Student's method for interpreting paired experiments as modified by Love (6). Odds that the infection percentage obtained with 6.8 mM sodium citrate is significantly greater than that obtained by water extraction are greater than 9999 to 1 in the 1934 experiments. Odds that the 3.4, 6.8, and 13.6 mM sodium citrate concentrations were significantly greater with respect to infections obtained than was the case with water extraction were 132, 3332, and 124 to 1, respectively, for the 1935 tests.

On comparing the infection percentage of the tests conducted in 1935 with those of the previous year, it is evident that some factor, yet unknown, entered that greatly reduced the amount of infection. This is especially true in the control tests in which water was the extracting solution. The infection percentage in the control tests in 1935 was only one-fourth that of the control tests conducted in 1934. This difference cannot be attributed to the leaf hoppers, for all used were reared in the insectary by the regular method for producing nonviruliferous leaf hoppers (9).

Although only 5 experiments were conducted using 13.6 mM, it appears that in these tests (1935) in which the virus content was extremely low the concentration of sodium citrate releasing the greater amount of virus may lie between 6.8 and 13.6 mM.

Despite the extremely low percentage of infection in the control tests of 1935, the infections, as shown by the average percentages obtained for the 3 lower concentrations of sodium citrate in these tests, are even more striking than those obtained in 1934. In the 1935 tests, increases in the percentage of infection of 180, 475, and 462 per cent for the 3.4, 6.8, and 13.6 mM concentrations, respectively, were obtained.

Sodium citrate in concentrations greater than 13.6 mM reduced the amount of infection below that of the control tests. This cannot be ascribed to its effect on the leaf hopper because leaf hoppers were unaffected by feeding upon sodium citrate concentrations as high as 54.4 mM. Mortality of the leaf hoppers was low during the feeding period and equally distributed over the controls and all the sodium citrate concentrations used.

In order to consider the virus-releasing ability of sodium citrate as a function of the amount of virus released by water, the foregoing experiments were placed in 4 groups, according to the percentages of infection obtained with water extractions. The first group (A) consisted of all experiments in which the infection percentage in the control tests was 10 or below. Group (B) contained those experiments in which the control tests ranged from 11 to 20 per cent infection. Group (C) contained those experiments in which the control tests ranged from 21 to 40 per cent. All experiments in which the infection percentage in the control tests was above 41 constituted the fourth group (D).

Within each group, the ratios of the average percentage of infection obtained by the various citrate concentrations to the average percentage of infection of the control tests are plotted against the citrate concentrations. The results of thus grouping the experiments are shown in figure 2.

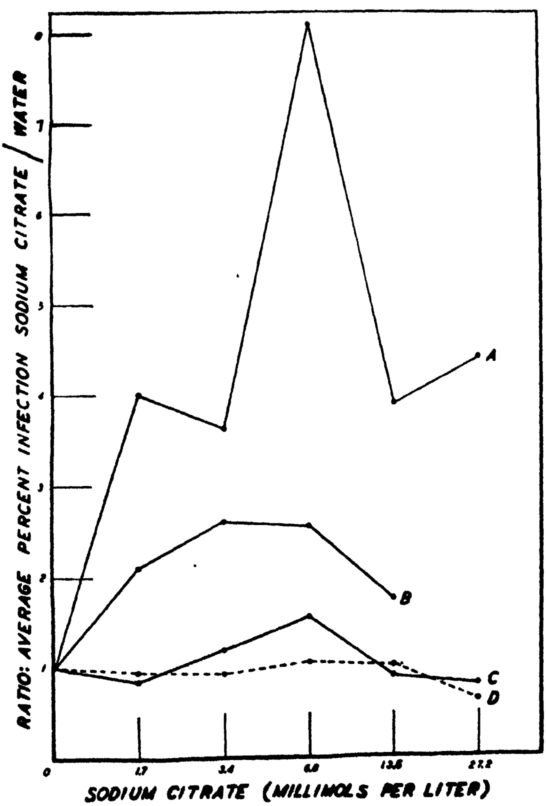


FIG. 2. Data from figure 1, showing ratios of infection percentages, sodium citrate to water, grouped as follows: A, infection in controls less than 10 per cent; B, infection in controls 11 to 20 per cent; C, infection in controls 21 to 40 per cent; and D, infection in controls 40 per cent or above.

Calculations (6) show that the infection percentages using 6.8 millimolar concentrations of sodium citrate were significantly greater than those for

water extractions in groups A, B, and C. The odds favoring 6.8 millimolar sodium citrate are greater than 9,999 to 1 for groups A and B, and 66 to 1 for group C. For group D, the two methods of extraction did not differ.

The increase in percentage of infection with sodium citrate extracts relative to water extracts becomes much greater as the amount released by water decreases. Where the virus is apparently so strongly adsorbed by the precipitate that water is only capable of liberating sufficient virus to give 10 per cent infection, sodium citrate is very effective in liberating the virus. In these tests, 6.8 mM sodium citrate liberated sufficient virus to increase the average percentage of infection 8-fold over that obtained with water. Of the 15 control tests of group A, 7 failed to give infection. In 4 experiments in which no infection was obtained in the control tests, 6.8 mM sodium citrate gave 50, 50, 30, and 20 per cent infection.

Group B, in which 10 and 20 per cent infection was secured from the water extract, the virus was still apparently adsorbed by the precipitate, since sodium citrate increased the percentage of infection 2.5-fold. It appears, from the experiments in which the control tests gave 21 and 40 per cent infection (group C), that the virus was adsorbed in those cases only to a limited extent. In this group, 6.8 mM sodium citrate increased the infection percentage only 1.5-fold above that of the controls. In those experiments in

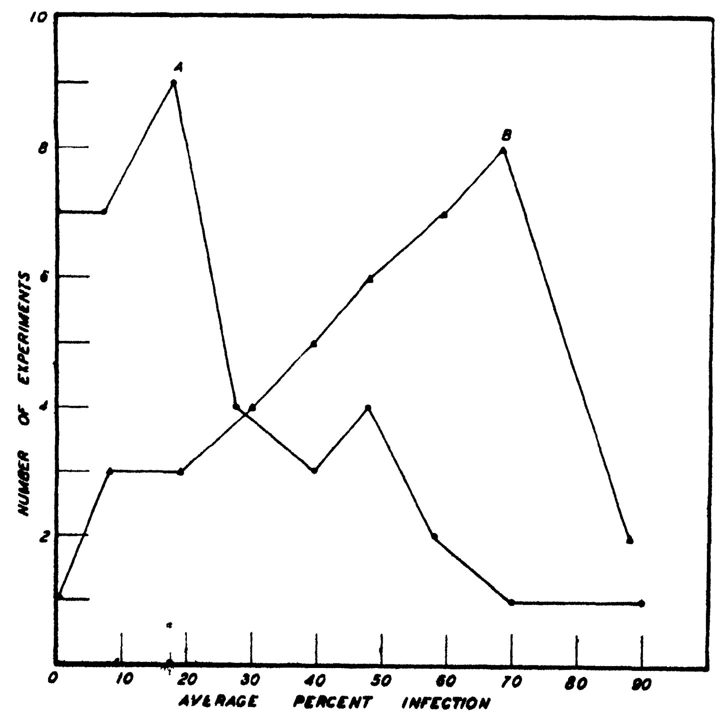


FIG. 3. Distribution of the experiments with respect to amount of infection obtained: A, experiments in which virus was extracted from the alcoholic precipitate with water; B, experiments in which sodium citrate was used to extract virus.

which water was capable of liberating sufficient virus to cause greater than 41 per cent infection (group D), no increase in infection was noted where sodium citrate was used as the extracting solution.

To point out further the effectiveness of sodium citrate in comparison with water as a releasing agent, the data of the experiments were separated as to extraction method and grouped according to the percentages of infection obtained. The groups were as follows: 0, 1 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, and 71 to 90 per cent. The average percentage infection of each group was then plotted against the number of experiments falling within each group (Fig. 3). Where water was used as the extracting solution, 55 per cent of the experiments fell in the first 3 groups, whereas only 18 per cent of the experiments in which sodium citrate was used as the extracting solution fell in these same groups and 59 per cent of the experiments fell in the 4 highest groups. There were 7 experiments out of a total of 38 conducted over a period of 2 years (a different source of virus for each experiment) in which no infection was obtained when water was used for extraction. Such results, if only water extractions had been used, would be taken to indicate that no virus was present in approximately 18 per cent of the experiments, but in these experiments the virus was demonstrated to be present with sodium citrate as the extracting agent. While water failed to give infection, infection with sodium citrate varied from 5 to 70 per cent, with an average of 26 per cent.

Effect of Hydrogen-ions on the Release of Virus by Sodium Citrate

Bennett (1) subjected the virus to various hydrogen-ion concentrations ranging from pH 2.4 to 9.1 by adding 1 volume of phloem exudate, containing the virus, to 4 volumes of buffered solutions. After the mixture stood 2 hours, a sample was removed for pH determinations and the remainder was fed to nonviruliferous beet-leaf hoppers 4 hours. Thus, the virus was subjected to the hydrogen-ion activity of the medium for a minimum period of 2 hours and a maximum period of 6 hours. He obtained no infection when the virus was subjected to a pH less than 3.4. Only 5 per cent infection was obtained at pH 3.4. In another experiment, pH values below 4.0 apparently inactivated the virus.

Bennett (1) also found that 4 volumes of juice of *Oxalis corniculata* L. inactivated the virus when mixed with 1 volume of juice from diseased beets (pH of mixture, 2.3–2.5) in about 30 minutes. The virus remained in contact with the juice for a definite period and then was precipitated with alcohol and removed from the presence of the acid by centrifuging.

A short exposure to a comparatively low hydrogen-ion concentration might not destroy the virus, but merely cause it to be adsorbed or fixed by the plant proteins in such a way that little or no virus would be liberated by extraction with water. Experiments, therefore, were conducted to compare the ability of sodium citrate and water to liberate virus that had been subjected previously to various hydrogen-ion concentrations. Using 1-ml.

aliquots of juice extracted from diseased beet leaves, various amounts of sulphuric acid were added to bring the pH to definite values. Each sample of treated juice was shaken and allowed to stand for 15 minutes. It was then precipitated and treated in the usual manner.

The beef-leaf juice was adjusted to a pH value of 4.7 in 5 experiments and to 4.1 in 7 others. Inasmuch as the amount of infection was in the same general range for both pH values, the data obtained are combined. Tests were made in 1934 and repeated in 1935. The results for the two years are shown in figure 4.

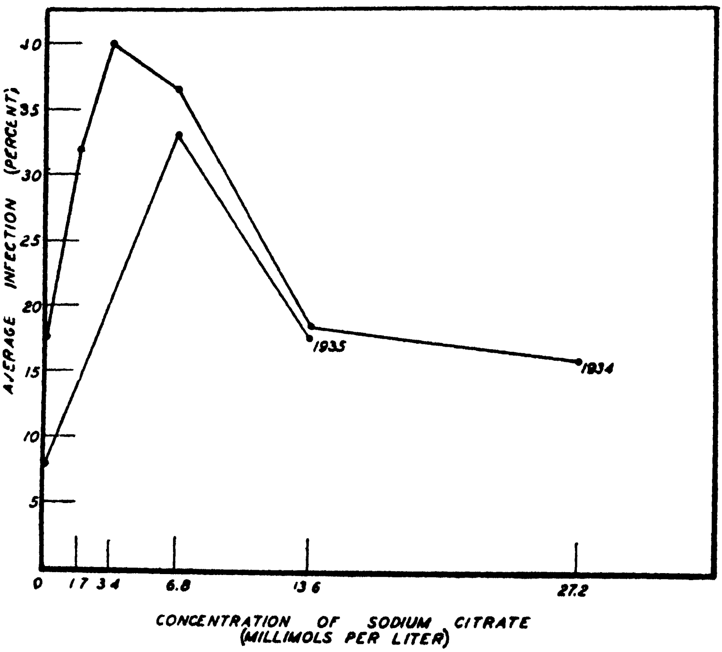


FIG. 4. Release of curly-top virus by various concentrations of sodium citrate from the alcoholic precipitate of beet-leaf juice adjusted to pH values of 4.1 and 4.7.

Here, as in figure 1, sodium citrate liberated more virus than did water. In these tests the 3 lowest concentrations appeared to be approximately equal in their ability to liberate the virus. It is evident that the exposure to pH 4.1 or 4.7 caused a reducion in the amount of virus extracted by water and that sodium citrate counteracted this effect, since the infections obtained reached levels comparable with those of former experiments.

Tests also were made to determine the liberation of virus from samples of juice from diseased beet leaves that had been subjected to higher hydrogen-ion concentrations. The data are presented in table 2. In Experiment 4, juice of Oxalis was used to adjust the pH. It is evident that exposures of the virus (in beet-leaf juice) to hydrogen-ion concentrations of pH 3.7, 3.4, and 2.2 the amount of virus extracted by water was decidedly below that shown for the nontreated juice (pH 6.2). When sodium citrate was used to extract the virus, the percentages of infection rose sharply, exceeding in

TABLE 2.—*Effect of certain hydrogen-ion concentrations on the virus in the expressed juice from beet leaves affected with curly top*

Experiment number	Reaction to which the plant juice was adjusted before precipitation	Infection secured from alcoholic precipitate following extraction with: Water	Sodium citrate at indicated concentrations: 3.4 mM	6.8 mM	34 mM
	pH	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	6.2 (normal)	50	70	50	
	3.7	0	60	55	
	3.4	15	80	20	
2	6.2	0	10		
	3.7	0	20		
	3.4	0	10		
3	6.2	30			25
	2.2	0			30
4[a]	6.2	55		70	
	2.5[b]			20	
	2.5[c]			60	

[a] Allowed to stand for 16 hours in the presence of the acid.
[b] Brought to pH 2.5 by the addition of Oxalis juice.
[c] An equal volume of 95 per cent alcohol and sufficient Oxalis juice was added to reduce the pH to 2.5.

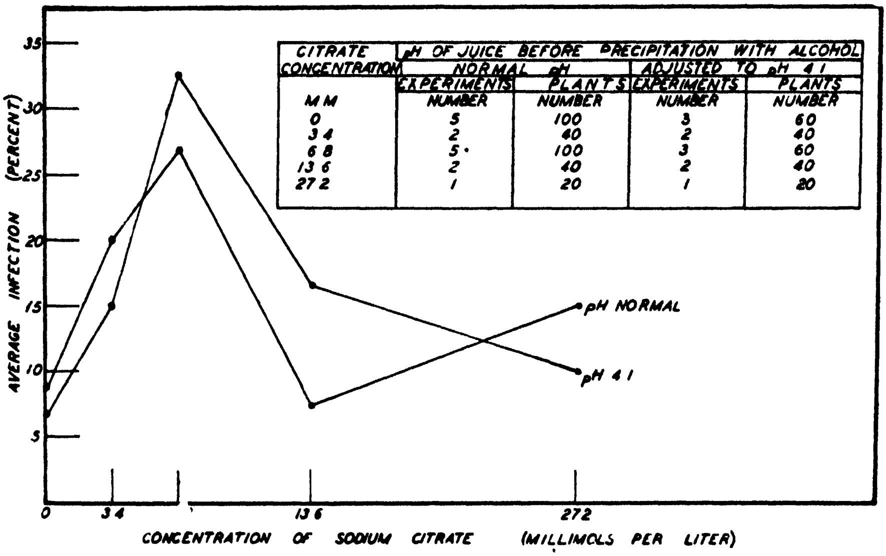
many cases the percentages obtained when the juice was not exposed to high hydrogen-ion concentrations. It is believed that the virus is not destroyed at the lower pH values but only fixed or adsorbed.

In another test the virus was subjected to 0.1 normal sulphuric acid for a period of 2 hours. Following this exposure, the virus was precipitated from 3, one-ml. aliquots of juice and washed in the usual manner. One of the precipitates was extracted with 6.8 mM sodium citrate. Fifteen per cent infection was obtained with this extract. The two remaining precipitates were resuspended in 2 ml. of 0.1 normal sulphuric acid and allowed to stand, with frequent shaking, for 2 hours. The acid was neutralized with 2 ml. of 0.1 normal sodium hydroxide and the virus was again precipitated with alcohol, washed, and dried in the usual manner. One of the precipitates was extracted with water, while the other was extracted with 6.8 mM sodium citrate. These extracts were tested for the presence of virus, with the result that no infection was obtained with water, whereas 10 per cent infection was obtained with sodium citrate. From this experiment, it appears that the virus is not destroyed by brief exposures to hydrogen-ion concentrations equivalent to approximately pH 1.

Release of Virus from Tomato-Plant Juice Adjusted to Various Hydrogen-ion Concentrations

Tests were made to determine if sodium citrate is capable of liberating the virus from the alcoholic precipitate of the juice of other curly-top-susceptible plants. Diseased tomato plants were frozen and the juice was

extracted and centrifuged. The virus was precipitated from 1-ml. aliquots of juice with alcohol, washed, and dried in the usual manner. The virus then was extracted with water (for the control test) and with the usual concentrations of sodium citrate. Tests also were made to determine if sodium citrate is capable of liberating the virus from tomato-plant juice that had been previously adjusted to a pH value of 4.1. The methods used in these experiments were the same as those previously described. The results of the experiments in which diseased tomato plants were used as the source of virus are summarized in figure 5.



CITRATE CONCENTRATION	pH OF JUICE BEFORE PRECIPITATION WITH ALCOHOL			
	NORMAL pH		ADJUSTED TO pH 4 1	
	EXPERIMENTS	PLANTS	EXPERIMENTS	PLANTS
MM	NUMBER	NUMBER	NUMBER	NUMBER
0	5	100	3	60
3 4	2	40	2	40
6 8	5	100	3	60
13 6	2	40	2	40
27 2	1	20	1	20

FIG. 5. The release of curly-top virus from alcoholic precipitate of tomato plant juice with water and with various concentrations of sodium citrate.

It appears that the same factors influencing the binding of the virus in the precipitate from beet-leaf juice are operative in the precipitates from juice of diseased tomato plants. The maximum amount of infection was obtained with 6.8 mM of sodium citrate when the tomato plant served as the source of virus.

The experiments illustrated in figure 5 were conducted in 1935 concurrently with those represented by the lower curve of figure 1. For some reason, in 1935, little virus was released from the alcoholic precipitate when water was used for extraction, regardless of the source of virus (Figs. 1 and 5). In the 1935 experiments sodium citrate (6.8 mM) was, however, capable of liberating sufficient virus from the alcoholic precipitate of either beet-leaf juice or tomato-plant juice to cause a 4-fold increase over that obtained with water.

Subjecting the virus in the tomato-plant juice to a hydrogen-ion concentration of pH 4.1 apparently did not greatly influence the amount subsequently released by either water or sodium citrate.

The Peptization of the Proteins in the Alcoholic Precipitate of Beet-Leaf Juice by Different Concentrations of Sodium Citrate

Gortner, Hoffman, and Sinclair (5) demonstrated that the protein complex of wheat flour could be peptized to different degrees when treated with different inorganic salt solutions. Sinclair and Gortner (8) studied the physico-chemical behavior of a pure protein (gliadin) when treated with neutral salt solutions. These investigators found that the amount of protein (gliadin) that will be peptized by a particular salt solution depends both upon the concentration of the salt solution and the amount of gliadin present.

Experiments were conducted to determine if any correlation exists between the amount of protein brought into solution by extracting the alcoholic precipitate from beet-leaf juice with the various concentrations of sodium citrate and the amount of virus released by the same concentrations.

Leaves from diseased beet plants were collected and frozen. The juice was expressed and the coarser particles allowed to settle. Two hundred ml. aliquots of juice were pipetted off the top, adjusted to pH 4.7, and sufficient alcohol was added to bring the concentration to 50 per cent. The mixture was allowed to stand overnight at room temperature, and then the precipitate was filtered off and washed once with 50 per cent alcohol.

The precipitates were ground in a mortar in the presence of small amounts of the extracting solution. Grinding was continued until the volume in each case was brought to 200 ml. Extraction was allowed to proceed at room temperature for 24 hours, then the extracts were filtered through paper pulp until a clear liquid was obtained. The amount of nitrogen in the filtrate was taken as a measure of the amount of protein peptized by the various concentrations of sodium citrate used. The data are shown in figure 6, together with the amount of infection obtained at the same concentrations.

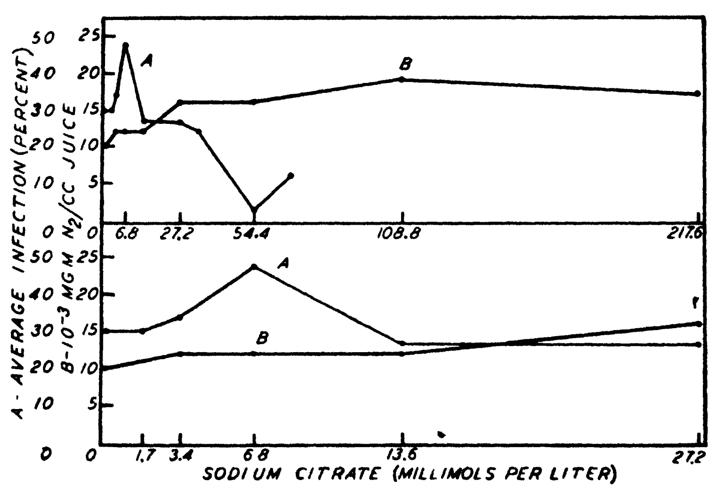


FIG. 6. Peptization of proteins (curve B) and amount of infection (curve A) obtained with the same concentrations of sodium citrate. Results at lower concentrations also plotted on larger scale.

It is noted that the maximum amount of infection was obtained at 6.8 mM sodium citrate, whereas the maximum amount of proteins were peptized by a concentration of 108.8 mM sodium citrate (Fig. 6). Obviously, the amount of protein peptized by the various concentrations of sodium citrate bears no direct relation to the amount of infection obtained.

DISCUSSION

The method developed by Bennett (1) for testing the presence of virus and, from this, obtaining an estimate of the amount present, although greatly improved over the method of Carter (3) and Severin and Swezy (7), is not completely reliable. Extraction of the alcoholic precipitates with water gives variable results, especially where the amount of recoverable virus is low. Extraction with sodium citrate gives more dependable results.

Inasmuch as all proteins exhibit electrokinetic phenomena (2), (4), it was postulated that the virus may be adsorbed under certain conditions on proteins or other substances in the colloidal state that are precipitated from beet-leaf juice along with the virus upon the addition of alcohol. If this were true, the alcoholic precipitate might contain large quantities of virus so completely adsorbed by the proteins that little or none of it would be liberated from the protein-virus complex by extraction with water. Experiments were undertaken in an attempt to develop a method that would release the virus and thereby increase the amount of infection.

The results of the experiments reported strongly suggest that some protein or other substance present in the juice of sugar beets, tomatoes, and probably other plants adsorbs the virus with such avidity that water is incapable of liberating it. It seems probable that when this adsorbing complex is saturated with virus and an excess of virus left free, then extraction of the alcoholic precipitate with water may give an indication of the amount of nonadsorbed virus present rather than the total amount present. Those experiments in which no infection was obtained when water was used for extraction may be accounted for by complete adsorption of the virus. When the same precipitates were extracted with sodium citrate, infection was obtained, which indicates definitely a release of the curly-top virus.

In attempting to explain the mechanism by which the virus is released from the plant protein, or other substances, probably colloidal in nature, the theory of Helmholtz, involving the presence of an electric double layer in the immediate vicinity of the colloidal micella, must be taken into consideration. It is known, for example, that different proteins under the same conditions may exhibit different properties, depending upon the magnitude and sign of the charge on the micellae. It is possible that the micellae of 2 proteins in the colloidal state under the same conditions may possess opposite charges. If the micellae of plant proteins possess a strong charge, they would attract and hold ions and micellae of the opposite charge. The principal force by which the curly-top virus is closely held by the plant proteins, or other substances in the precipitate, may be allied to these electrokinetic phenomena.

The effect of certain ions on the electrophoretic mobilities and the sign of the charge carried by proteins is well demonstrated by Tiselius (10). The addition of a non-electrolyte, such as water or sucrose, would neither reduce nor increase the charge set up at the electric double layer; consequently, it would have little effect upon the adhesive forces binding the virus to the plant proteins or upon the forces holding the particles together. The addition of an electrolyte would, however, affect profoundly the properties exhibited by the different micellae in the system.

SUMMARY

Preliminary experiments indicated that curly-top virus may be so intimately tied up with the plant proteins and other substances precipitable by alcohol that water is unable to release sufficient quantities of virus to give consistent amount of infection. Sodium citrate, however, which ranks at the top of the lyotrophic series, proved very effective in releasing the virus.

Maximum infection was obtained when the alcoholic precipitate was extracted with 6.8 mM sodium citrate. Concentrations greater than this amount generally reduced the percentage of infection.

It appears that where the extractable virus content of the plant juice is low, as shown by the percentage infection obtained in the control tests, the sodium citrate method of extracting the virus is far superior to the method in which water alone is used, and a part of the variability in results avoided.

When the pH of the extracted beet leaf juice containing the virus was reduced below 4.1, prior to precipitation with alcohol, limited infection was obtained if water was used for extraction. The virus, however, is not irreversibly inactivated at these pH values or at values as low as pH 2.2, because infection was obtained by extracting the alcoholic precipitates obtained after such exposures with sodium citrate. The virus apparently was only fixed in such a way that water is incapable of liberating it from the precipitate.

It appears that the same factors that fix or adsorb the virus in the juice from diseased beet leaves may also be operative with the virus in juice from the tomato plant, since the maximum percentage of infection with both plant juices was obtained after extraction of the precipitates with 6.8 millimols of sodium citrate.

Subjecting the virus in the tomato plant juice to a hydrogen-ion concentration of pH 4.1 had no definite effect on the amount subsequently released by water or sodium citrate.

The hypothesis is offered that these results may be explained on the basis of electrokinetic effects produced by the sodium citrate.

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THE COMPARATIVE IMPORTANCE OF LEAVES AND TWIGS AS OVERWINTERING INFECTION SOURCES OF THE PEAR LEAF-BLIGHT PATHOGEN, FABRAEA MACULATA

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INTRODUCTION

Leaf blight of pear and quince is a common disease in the eastern half of the United States, and at one time or another since 1854 (5) or earlier it has been reported from many of our States (6, 8, 12, 13, 14, 15, 17, 18, 19, 22, 24, 25, 29, 30, 35, 36, 38, 39, 40, 41, 47, 48, 49, 50). It is often a serious disease of pears and quinces grown in poorly sprayed orchards and in home gardens. It has also been reported from Canada (1, 21, 27, 31), and is well known in New Zealand (7, 20), Australia (9, 26), South America (16), South Africa (10, 11), Asia (28, 45, 51), and in Europe (23, 32, 33, 37, 42, 43, 46).

It has also been described on *Sorbus* sp. (2, 8), *Amelanchier* sp. (49), *Aronia* sp. (37), *Cotoneaster* sp. (33), and *Photinia* sp. (46).

OVERWINTERING OF THE ORGANISM ON DISEASED LEAVES

Since Atkinson (3, 4) collected and described the type material of *Fabraea maculata* (Lév.), the importance of the ascigerous stage in the dissemination of the disease from year to year has been emphasized (34, 42, 43). Although pathologists (7, 12, 39, 44) know that the ascigerous stage is not very common, none has made any attempt to find out whether or not this stage is essential to the overwinering of the fungus. For several years the writers (39) have made a diligent search for perithecia and acervuli on overwintered leaves in Maryland and Missouri, but have found no perithecia and only a few conidia. To determine whether or not fallen diseased leaves were of importance in carrying the pathogen over from season to season, several sets of experiments were carried out in Maryland and Missouri.

In one set of experiments diseased leaves were taken from beneath diseased trees and placed under trees that had been kept free of the disease by spraying. In collecting diseased leaves from beneath certain trees it was possible, because of the absence of diseased neighboring trees, to remove all the diseased leaves from under and around the experimental trees. In this experiment, therefore, trees not previously diseased were exposed, and those previously diseased were not exposed to diseased leaves. If the disease developed in the first case, then diseased leaves were responsible. If it developed in the second, diseased leaves were not responsible. The trees used in this experiment were not sprayed during the following growing season. Experiments of this type were conducted during the 1935 and 1936 seasons in Maryland and Missouri. Tables 1 and 2, respectively, show the results obtained at Beltsville, Md., and at the Missouri stations in 1935 and 1936. Fruit infection was negligible on the Beltsville trees, exposed to diseased leaves for the first time, but was plentiful both on trees that were deprived of diseased leaves and on trees under which the diseased leaves were allowed to remain. At the Missouri stations primary leaf-blight did not occur on trees deprived of their bark cankers and diseased leaves, and infections were delayed on trees deprived of the bark cankers but exposed to diseased leaves. At Beltsville one tree previously free from the disease was exposed to diseased leaves for 2 years, but only a very few leaves became infected.

TABLE 1.—*Relation of diseased leaves beneath disease-free pear trees to incidence of leaf blight in such trees and also the effect of removal of blighted leaves from beneath diseased trees on the recurrence of leaf blight in those trees*

No. of trees	Treatment	Year	Fruits counted	Diseased	Diseased
			Number	*Number*	*Per cent*
2	Diseased leaves placed under	1935	794	7	0.9
4	do	1936	2974	28	0.9
1[a]	do	1935	1287	5	0.4
		1936			
1	Diseased leaves taken from	1935	793	33	4.16
1	do	1936	2252	695	30.9
1[b]	do	1935	725	461	63.6
		1936			
4	Diseased leaves left under	1935	5939	2021	35.6
4	do	1936	9781	2687	37.5

[a] Diseased material placed beneath the same tree during two seasons.
[b] Diseased material removed from beneath the same tree during two seasons.

During these 2 years of studies on the effect of overwintered leaves on the carryover of the leaf blight, other observations were made that confirm the results of these experiments. Several thousand pear seedlings, severely affected during their previous growth history by the leaf-blight organism, were moved in the winter of 1934 from the Arlington Experiment Station grounds in Virginia to a site at the United States Horticultural Station at Beltsville, Md., where no pear trees previously had been grown. Very little leaf material accompanied their transfer. The following spring, soon after

the new leaves appeared on these seedlings, typical leaf blight developed throughout the planting and caused severe early defoliation. Bark cankers developed plentifully during the season, and in the spring of 1936 outbreaks of primary leaf blight were traced back to these cankers. In all cases examined one or more cankers could be found just above the group of infected leaves.

Following the mild winter of 1936–37 at Beltsville, Md., many diseased leaves were found harboring viable conidia during the growing season of 1937. A number of these were placed in several wire baskets among presumably susceptible pear and quince leaves during rainy periods, and in each case a few typical leaf-blight lesions were produced on leaves just below the diseased leaves. In no case were lesions formed on leaves above the sources of inoculum. From this evidence, it appears that the few infections that developed were caused by conidia and not by ascospores, because if ascospores had been the responsible agent the infections would have occurred more generally and on leaves above as well as below the source of inoculum. It is possible, of course, that the lesions may have developed from chance infections due to conidia and ascospores in the orchard environment. It is unlikely, however, that this was true, since the observed lesions always were located just below the diseased leaves. Furthermore, despite extensive search, no asci have been found in diseased leaves collected in this orchard. On the other hand, viable conidia were known to be present on these diseased leaves. Since the leaf-phase experiments appear to demonstrate that diseased leaves are not commonly of much importance as overwintering infection sources, studies on the fruit and bark phases of the disease were initiated.

OVERWINTERING OF THE ORGANISM ON DISEASED FRUITS

The fruits of pear are severely affected by the leaf-blight organism and many of the diseased fruits are discarded and allowed to remain on the ground. Since lesions on these fallen diseased fruits persist for a long time, it was thought that possibly the pathogen might overwinter in them. Conidia have been found on diseased fruits during the winter months, but no signs of an ascigerous stage have been observed. During the fall of 1935 and 1936 diseased fruits were collected and placed under trees that had previously been free from the disease. None of these trees became diseased during the two seasons. The diseased fruits slowly disintegrated during the winter and at the time the disease usually becomes prevalent among the newly formed leaves in the spring, the old lesions in the fruit were hardly recognizable. No viable conidia as determined by germination tests, were ever observed on overwintered fruit at that season.

OVERWINTERING OF THE ORGANISM IN BARK CANKERS

Sorauer (42), who was probably the first to describe the bark cankers, was of the opinion that they furnished a possible overwintering source of conidia. Although others (6, 11, 12, 14, 18, 23, 26, 44) have pointed out the

possibilities of this source of inoculum, none have demonstrated the real importance of bark cankers and how conidia are produced in them.

During the preliminary stages of these investigations, which were primarily concerned with control, it was observed that the location of the first infected leaves differed from that which would be expected if the source of spores was confined to overwintered diseased leaves. The primarily infected leaves on check (unsprayed) trees were always localized in inverted-cone-shape areas, directly below bark cankers that were yielding viable conidia. Never was the infection general except at a time of the season when conidia were being disseminated from the primary lesions. The disease usually became visible on the young leaves by the middle of May, but viable conidia were found in acervuli on bark cankers of the previous season before any leaf infections were visible. Only a relatively small percentage of the observed bark cankers appear to become active during the following spring, but occasionally a few conidia are obtained from cankers that are 2 years of age.

After the new leaves become infected and conidia are produced, bark cankers resulting from these conidia may be formed on current-year growth at any time during the remainder of the growing season, provided the environmental conditions are suitable and the bark tissues are in an immature condition. Bark cankers may be formed in the early spring or even late in the summer, and both early- and late-formed bark cankers may be active the following spring. Most of the cankers, however, appear to be active for only a relatively short period, and the great majority of them not only do not produce conidia but remain very small or are healed out. Many small cankers occurring on twigs and watersprouts do not develop to the stage where they produce acervuli. At times, however, the infections are more deep-seated, and the fungus may be readily isolated from cankers of this type. Several cankers may coalesce to form lesions of several centimeters in extent from which the pathogen may be isolated during a period of several months. These cankers are usually black and are never of a regular size or outline; usually the conidia escape through cracks in the epidermis above the surface of the acervuli, probably caused by the increase in girth of the stems.

During 1935, 1936, and 1937 a special study was made of the canker phase of the disease, since studies of the leaf phase had indicated that this was not the important means of overwintering of the pathogen.

In the fall of 1935, at Springfield, Mo., many cankers were tagged for observation, some of which were formed early and some late in the 1935 growing season on both Kieffer and Garber trees. Collections were made during the fall and winter of 1935 and the spring of 1936. The collected material was fixed in formalin-acetic acid-alcohol, embedded in celloidin, sectioned, and stained with Delafield haematoxylin-eosin and with Orseillin BB. A series of sections, usually 10 μ in thickness, was cut from cankers collected throughout the fall, winter, and spring.

All the cankers are walled off from the healthy cortex by a layer of wound cork 5 to 10 cells deep. The fungus seldom penetrates very deeply into the

cortex and remains between the cells at all times. The intercellular mycelium appears always to be made up of short cells, and it is not certain just how the pathogen obtains its nutrition from the host cells. The sections do not show that the fungus possesses haustoria or extends beyond the layers of cork tissue. These observations explain why the cankers are generally superficial and are sloughed off as the twigs and branches become older.

In cankers collected during October, November, and December mycelium is present between the cells of the host, but no acervuli are formed. During January, February, and March changes take place that lead to the formation of the acervuli.

The acervulus is formed beneath the epidermis of the host by an apparent massing of mycelial strands growing out from between the cells of the host. As the acervulus increases in size, pressure is exerted on the epidermis of the host, forming blisters that are visible to the naked eye. As the acervulus matures, the characteristic conidia are formed beneath the epidermis of the host. A few of the acervuli bear conidia in March, but during April and May the great majority of them mature and the conidia are exposed by a breaking away of the thin epidermal layer of the host.

Supplemental to the collection and fixation of cankered material during the winter and early spring of 1935–36, pure culture isolations were made from similar material at various times. In nearly all cases the pathogen was isolated without difficulty.

In 1936 and 1937 similar experiments were conducted at Springfield and Mountain Grove, Mo., to find out whether or not bark cankers are infection sources in the spring. Trees pruned of their 1935 wood and not exposed to overwintered diseased leaves were caged with muslin. Trees not pruned of their 1935 wood and not exposed to overwintered diseased leaves were also caged with muslin. Noncaged trees, pruned and not pruned of their 1935 wood, were exposed to overwintered diseased leaves, and similar trees were

TABLE 2.—*Leaf infection from conidia produced from overwintered cankers on pear twigs and watersprouts at Mountain Grove, and Springfield, Missouri*

No. of trees	Treatment	Date of first observed infection in 1936	Period of maximum infection in 1936
2	Caged, pruned. Overwintered leaves removed	Not infected	
2	Caged, nonpruned. Ooverwintered leaves removed	May 7–8	June 10–20
4	Noncaged, nonpruned. Overwintered leaves left at base of trees	May 7–10	June 8–16
4	Noncaged, nonpruned. Leaves removed from base of trees. Ground scraped	May 9–12	June 10–15
4	Noncaged, pruned. Overwintered leaves left at base of trees	May 20–21	June 10–15
4	Noncaged, pruned. Leaves removed from base of trees. Ground scraped	May 27–June 1	June 10–15

not exposed to overwintered diseased leaves. The results of these experiments are shown in table 2. The experimental trees were known to have been heavily cankered in 1935, but nothing was known about their 1934 disease history. It is clearly shown that the removal of the 1935 bark cankers was responsible for a lack of the disease where the tree was protected by muslin from chance infections. It also is clearly shown that trees deprived of their diseased overwintered leaves, but not deprived of their 1935 cankers, became infected as early as those not deprived of their overwintered diseased leaves and 1935 wood. This indicates again that overwintered diseased leaves are not the overwintering agency. Noncaged trees deprived of their 1935 wood, either with or without the overwintered diseased leaves left at their bases, became infected much later than those exposed to overwintering bark cankers. These probably were chance infections and clearly indicate again the importance of the 1935 wood in causing the early infections. The same results were obtained at Mountain Grove and at Springfield, Mo., but the dates of infection differed slightly between the two stations. This accounts for the two dates appearing in the second column of table 2.

In 1937 two of the trees used in the 1936 experiments were studied further to find out whether or not the bark cankers formed during 1935 were still active during the 1937 season. From one of these all the 1935 and 1936 wood was removed; from another, all the 1936 wood was removed. Both trees were caged and all the leaves were removed from beneath both of them.

During the 1937 season, foliage infection, slight to begin with, developed on the tree deprived of its 1936 wood but not of its 1935 wood. No infection developed on the tree deprived of its 1935 and 1936 wood. This apparently indicated that the organism overwintered the second year in cankers on the 1935 wood. Table 2 indicates that this was not so in the case of cankers studied during 1935. Since we have observed that conidia remain in cankers during a great part of the season and also occasionally during the early part of the next season, it may be possible that chance infections may arise from this source during the second season after the cankers are formed. We have observed several times that slight infections develop in trees that have previously been kept clean for a season. It is possible that these infections arise from conidia that overwinter in the old established cankers. If the mycelium overwintered during the second winter in these old cankers, one would expect that the initial infections that arise from these sources the following spring would be of a major character. Since the infections are always slight, it is believed that only previously formed conidia survived the second season.

Spore trapping experiments also were conducted for the purpose of finding out when and under what conditions conidia were being disseminated from the bark cankers. It was found that, starting soon after the middle of April and continuing as late as the first week in June, 1936, conidia were being washed from the mature bark-canker acervuli during each rainy period. The greatest dissemination occurred during late May and early June, but a few mature conidia could be found in the cankers very late in the summer

and in the following spring. At the same time, in 1936, spore-trapping experiments with diseased overwintered leaves showed neither ascospores nor conidia to be present. During the spring of 1937 asci were absent but conidia were common on overwintered diseased leaves. This may be attributed to the mild winter of 1936–37.

SUMMARY

Leaf blight of pear and quince, caused by *Fabraea maculata* (Lév.) Atk., is a common disease in the middle-western and eastern parts of the United States and has been reported from many of our States. It is also common in many countries and attacks various pomaceous hosts.

While the ascospore stage has been observed infrequently by others, it has not been found on overwintered pear leaves under Maryland and Missouri conditions.

In Maryland and Missouri, conidia have been found to survive the winter on diseased leaves but not on diseased fruits. Occasionally, they overwinter in bark cankers.

Experiments have shown that overwintered conidia on diseased leaves may cause spring infections, but the evidence accumulated during several years indicates that these are not of importance in spreading the disease.

In Maryland and Missouri it has been found that the leaf-blight pathogen overwinters principally as mycelium in cankers formed in the bark.

The mycelium in the overwintering cankers forms acervuli during the late winter months, and, during early spring, conidia are produced in the acervuli and, escaping through cracks in the bark, infect the unfolding leaves.

Spore trapping experiments show that the conidia which are produced in the spring are disseminated from the cankers over a period of several months.

Conidia, escaping from the bark cankers, are washed downward through the tree, and cause in the leaves beneath the cankers cone-shape tracts of infection.

Conidia escaping from the leaf lesions may cause infections on the young bark of the growing stems any time during the growing season. In some of these bark infections the mycelium overwinters, and the life cycle of the pathogen is completed when conidia are produced in them the following spring.

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FURTHER STUDIES OF CRINKLE LEAF, A DISORDER OF COTTON PLANTS PREVALENT IN LINTONIA AND OLIVIER SILT LOAM SOILS OF LOUISIANA

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INTRODUCTION

In a previous publication[3] crinkle leaf, a new disease of cotton in Louisiana was described. It was pointed out that the disease was first observed in Louisiana in 1934 in certain areas of Lintonia and Olivier silt loam soils, and that in 1937 it was observed again in 4 additional fields in East Baton Rouge Parish, the disease occurring in a severe form in one of these fields with damage estimated at about 10 per cent.

It was further shown that the disease occurred on several varieties of cotton, that it is not hereditary, and that attempts to transmit it to healthy plants by grafting and budding and by injecting sap collected from various parts of affected plants were unsuccessful. Since the above-mentioned inoculation experiments demonstrated fairly conclusively that crinkle leaf is not a virosis, notwithstanding the similarity of some of the symptoms to those of viroses of other plants, and it was decided to make a study of certain soil factors—toxic substances or deficiencies that might be responsible for the disease. In preliminary experiments previous cultural and fertilizer practices of affected areas were studied and the pH and water-soluble manganese

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of the soil of affected and nonaffected areas were determined. Later, in both field and pot experiments, nutritional and toxicity studies also were made in an attempt to control and to induce the disease. These observations and experiments form the basis of this report.

PREVIOUS CULTURAL AND FERTILIZER TREATMENT OF AFFECTED AREAS

At the old experiment station in Baton Rouge, where the malady was first observed by H. B. Brown in 1934, the soil had been in cultivation for many years and had been fertilized for the 1934 cotton planting with 200 pounds of nitrate of soda, 300 pounds of superphosphate, and 50 pounds of muriate of potash per acre. At Pride, Louisiana, where the disease appeared in a severe form in 1937, the soil also had been in cultivation many years and had received for the 1937 crop 400 pounds per acre of a commercially mixed 4–12–4 fertilizer in addition to a ton per acre of barnyard manure. A striking feature of the soil of affected areas during the growing season was observed. It exhibits a moist, brownish surface discoloration after being exposed to rain and aeration for several days, giving the appearance of having received a large application of nitrates or some other deliquescent salt. This condition was lacking in areas where the cotton grew normally.

THE pH, WATER-SOLUBLE MANGANESE, AND WATER-SOLUBLE SALTS OF AFFECTED AND UNAFFECTED AREAS, AND TYPE OF PLANT GROWTH FOLLOWING CERTAIN SOIL TREATMENTS

A comparison of the pH of affected and unaffected areas showed without exception that the soil of the affected area had a lower pH. A comparison was then made of the water-soluble manganese and water-soluble salts and it was found that soil from the affected areas yielded approximately 4 times as much manganese as was obtained from the unaffected. More water-soluble salts and nitrates also were found in the affected areas (Table 1).

TABLE 1.—*Effect of different treatments of soil on the development of crinkle leaf in the field and in pot cultures*

Soil from	Treatment	pH	H_2O soluble Mn.	H_2O soluble salts	NO_3 N	Type of plant growth
			p.p.m.	*p.p.m.*	*p.p.m.*	
Affected field	400 lbs. 4–12–4 1 ton barnyard manure	5.0	46.4	580	90.9	Crinkle-leaf plants or none at all
Unaffected field	Same	5.5	11.9	440	55.5	Normal plants
Affected field in pots[a]	Same	5.1	52.3	. . .	.	Crinkle-leaf plants
Affected field in pots	Same plus 1 ton $CaCO_3$	6.4	0.8	...	..	Normal, healthy plants

[a] 8 kilos per pot.

FIELD AND POT EXPERIMENTS

The preliminary determinations of the pH and soluble manganese of the soil from the affected areas indicated that these factors were closely correlated with the disease. Therefore, a number of treatments were made on June 21 at Pride, Louisiana, to single affected Deltapine cotton plants in the field for an indication of a suitable control. Included in these preliminary tests were:

Treatment	Rate per acre lbs.
Calcium nitrate	200
Superphosphate	400
Potassium chloride	100
Sulphate of potash—magnesia	100
Calcium sulphate	1500
Calcium carbonate	1500
Calcium carbonate plus N.P.K.	1500
Copper, zinc, and boron solution to yield 1 p.p.m. each.	

Subsequent readings made later in the season showed that calcium carbonate and calcium carbonate plus N, P, and K gave some recovery. Later treatments, where the cotton was planted July 20 in 5-foot-row sections, showed that 3000 pounds of calcium carbonate broadcast and 500 pounds in the drill gave healthy seedlings.

With this indication, a series of pot-culture experiments were set up in which a better study could be made on seedlings. The results showed that calcium, magnesium, and potassium carbonates, equivalent to a rate of 1000 pounds per acre or more of calcium carbonate, were correctives for the disease (Fig. 1), the plants remaining healthy for a period of approximately 60 days, while the plants grown in nontreated soil from an affected area readily developed crinkle-leaf symptoms. Calcium sulphate, at the rate of 1000 pounds per acre in pot experiments, gave no control of the disease.

As a result of the above investigation it was apparent that the hydrogen-ion concentration or some substance made soluble by the high acidity was responsible for the disease. To test this possibility a series of pots was set up with river-washed sand[4] planted in duplicate to Deltapine cotton. All pots were adjusted to pH 5.0, including the controls. After the seedlings had formed true leaves, increasing amounts of manganous sulphate were added to some of the pots, 0.12 to 0.16 grams manganese per pot being the two highest increments, and increasing amounts of aluminum sulphate to the remainder. Hartwell and Pember's solution was added at intervals as needed. It was soon evident that insufficient amounts of manganese and aluminum had been added, so the amounts of manganese were increased to 0.2 and 0.5 grams manganese per pot for the 2 highest increments. What appeared to be the symptoms of crinkle leaf began to develop in about 22 days in plants that received the second treatment of manganese. The plants in the pots receiving moderate to large amounts of aluminum (0.08 to 0.32 grams per pot) remained normal or died without showing any symptoms of

[4] Each pot contained 7 kilograms.

FIG. 1. Deltapine cotton plants growing in Olivier silt loam. Left, soil from an area affected with crinkle leaf and treated with 50 pounds per acre of manganese sulphate, center, soil from an affected area, nontreated, right, soil from an affected area and treated with 1000 pounds per acre of calcium carbonate. Plants photographed after 60 days' growth.

TABLE 2.—*Effect of treating cotton seedlings in sand culture with manganese*

Sand culture 7 kilos per pot	Treatment[a] per pot	pH	H_2O[b] soluble Mn recovered	Plant growth after 25 days
			p.p.m.	
1	No Mn	5.1	None	Normal, healthy plants
4	0.3 gram Mn[c]	5.0	27.2	Slightly chlorotic showing leaf margin injury
8	0.7 gram Mn	5.0	41.7	Chlorotic, crinkling, flecking and retarded growth
9	1.0 gram Mn	5.2	74.8	Chlorotic, crinkling, flecking and retarded growth

[a] Hartwell and Pember's nutrient solution was added as needed to maintain rapid growth.
[b] Basis oven-dry sand.
[c] Supplied from manganous sulphate.

the disease. The concentration of the manganese finally was increased to 0.3 and 0.6 grams per pot in the 2 highest treatments originally used, and a fair reproduction of the symptoms was obtained. However, the affected plants were not dwarfed, due, no doubt, to the late application of sufficient manganese. Finally, a second set of sand cultures was set up and planted. At this planting a range of concentrations of manganese were added that was expected to cover the point at which typical symptoms could be obtained. All pots were again adjusted to pH 5.0, including the controls. The results obtained, together with the concentrations of manganese employed, are shown in table 2. In this experiment symptoms of crinkle leaf appeared in 12 days from the date of planting and within 25 days typical symptoms of the disease were produced (Fig. 2).

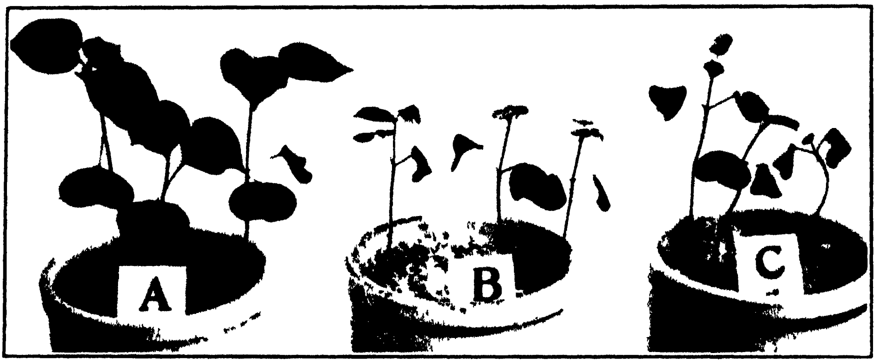

FIG. 2. Deltapine cotton plants grown in sand cultures with Hartwell and Pember's solution and varying amounts of manganese. A. Full nutrient solution only. B. Full nutrient solution plus 0.6 gram manganese. C. Full nutrient solution plus 1 gram manganese. Photographed after 25 days' growth.

DISCUSSION AND SUMMARY

As a result of experiments herein reported, it is indicated that the disease of cotton known as crinkle leaf and prevalent in certain Lintonia and Olivier silt-loam soils in Louisiana is associated with high acidity, calcium deficiency, and manganese toxicity.

Although the disease is readily corrected by calcium and other basic carbonates, it is not caused by a deficiency of calcium *per se*. In pot and field experiments calcium was supplied in the form of calcium sulphate in amounts sufficient for nutritional requirements, yet it had no effect in controlling the disease. This, very probably, is because of the fact that the hydrogen-ion concentration of the soil in the pots treated with the calcium sulphate remained essentially unchanged (pH 4.8 to 5), thus allowing manganese to remain in solution and cause the toxic effect as described for crinkle leaf. At the higher pH produced by treating the soil with the basic carbonates, calcium, magnesium, and potassium carbonate, the manganese apparently is precipitated and, accordingly, the plants remain healthy. The further evidence that soil from areas in Louisiana affected with crinkle leaf have a

consistently lower pH and contain significantly larger amounts of manganese than soil from healthy areas indicates that the disease is attributable to manganese toxicity. Moreover, reproduction of its typical symptoms in cotton plants in sand cultures by the addition of increasing amounts of manganese sulphate, as reported herein, strengthens this viewpoint.

LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

PATHOGENICITY OF CULTURE-REARED SPECIMENS OF THE BUD-AND-LEAF NEMATODE AND THE SUSCEPTIBILITY OF DIFFERENT STRAWBERRY VARIETIES

J. R. CHRISTIE

(Accepted for publication April 25, 1938)

THE PATHOGENICITY OF CULTURE-REARED SPECIMENS

In a former paper[1] it was pointed out that the southern strawberry strain of *Aphelenchoides fragariae* (Ritzema Bos, 1891) can be successfully reared on agar cultures. Before using this procedure in the investigation of summer dwarf it was deemed advisable to determine if these culture-reared specimens, when transferred to strawberry plants, would produce typical symptoms of the disease.

In the following tests, unless otherwise noted, the specimens used to inoculate the plants were washed from the cultures with water, concentrated in a small amount of fluid by centrifuging and transferred to the crown of the plant with a dropper. In all cases the nematode population employed had been on culture continuously since September 13, 1935. Strawberry plants of the variety Blakemore were used throughout.

Greenhouse Experiment.—In this test, conducted in a greenhouse at Washington, D. C., 45 potted strawberry plants were arranged in 3 lots of 15 plants each, which, for convenience, are designated A, B, and C. Lots A and C were inoculated with specimens of *Aphelenchoides fragariae* direct from the cultures. Lot B was inoculated with culture-reared specimens that were surface-sterilized for 30 minutes in a 1 to 2000 solution of mercuric chloride. The method of sterilizing was the same as that given in a former paper.[1]

Lots A and B were inoculated May 22, 1936. Disease symptoms appeared in about 6 weeks and the plants were examined microscopically between July 30 and August 3, or after 69 to 73 days. The plants in lot C, inoculated July 17, began to show symptoms in about 4 weeks and were examined microscopically on August 20, or after 34 days. The results of this experiment are shown in table 1. Forty noninfested plants served as controls, 10 of

[1] Christie, J. R., and Louise Crossman. Notes on the strawberry strains of the bud and leaf nematode, *Aphelenchoides fragariae*, I. Helminthol. Soc. Washington Proc. 3: 69–72. 1936.

which were examined microscopically on August 11. None of the control plants developed recognizable symptoms, and specimens of *Aphelenchoides fragariae* were not found in those examined. In all 3 lots symptoms were moderately pronounced (Fig. 1). The plants in lot B that were inoculated

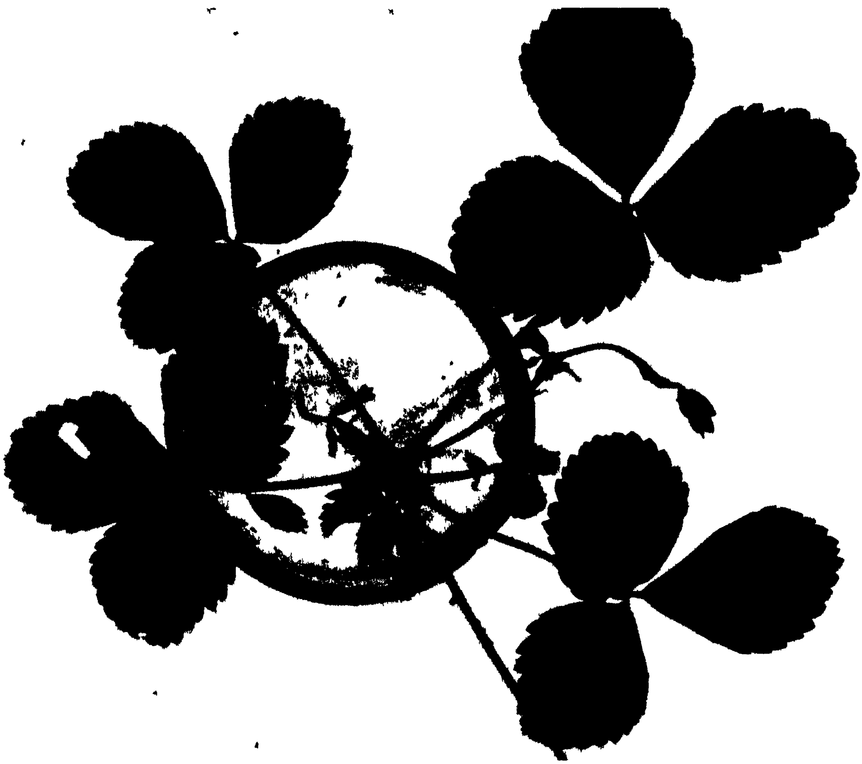

FIG. 1. Typical symptoms of summer dwarf on strawberry plant inoculated with culture reared specimens of *Aphelenchoides fragariae* in a greenhouse.

with surface-sterilized specimens showed no appreciable difference from the others.

TABLE 1.—*Development of symptoms of summer dwarf on strawberry plants inoculated with culture-reared specimens of Aphelenchoides fragariae and grown in the green house*

Condition of plants	Lot A		Lot B		Lot C	
	No. infested	No. not infested	No. infested	No. not infested	No. infested	No. not infested
With unquestionable symptoms	12	0	11	0	10	0
With possible symptoms	0	0	0	1	2	1
Normal	0	3	3	0	2	0

Field Experiment.—This test was conducted in the open field at the North Carolina Coastal Plain Station, Willard, N. C. On January 13, 1936, 78

strawberry plants were set in 3 rows of 26 plants each, which, for convenience, are designated A, B, and C. For row A the nematodes were washed from the cultures with water and concentrated in a small amount of fluid by centrifuging. After sundown on July 22, about 2 or 3 drops of this fluid were placed in the crown of each plant. For row C the nematodes were washed from the cultures with water but were not concentrated by centrifuging. During the early forenoon of July 23 about 6 or 8 cc. of this water were placed in the crown of each plant. A considerable part of the fluid ran into the soil at the base of the plant. The plants in row B (the middle row) were not inoculated and served as controls. Data were secured on August 25, September 21, and October 20 and are summarized in table 2.

TABLE 2.—*Development of symptoms of summer dwarf on strawberry plants inoculated with culture-reared specimens of Aphelenchoides fragariae and grown in the open field*

Condition of plants	No. of plants Row A			No. of plants Row B			No. of plants Row C		
	Aug. 25	Sept. 21	Oct. 20	Aug. 25	Sept. 21	Oct. 20	Aug. 25	Sept. 21	Oct. 20
With very pronounced symptoms	9	9	15			1			9
With moderate but unquestionable symptoms	8	14	8			1	3	18	12
With possible symptoms	4					2	2	4	2
Normal	4	1	1	25	25	21	21	3	1
Dead	1	2	2	1	1	1		1	2

In general, symptoms were much more severe than in the greenhouse test. Although the rows were only about 3 feet apart, it was not until October 20 that symptoms appeared in row B, presumably through spread from the adjoining rows. The plants were not examined microscopically.

The nematodes used in the 2 preceding experiments had been maintained continuously on cultures for from 8 to 10 months, and those used in the experiment discussed in the following section had been maintained on culture for 21 months. These experiments appear to demonstrate that rearing this strain of *Aphelenchoides fragariae* on culture does not reduce its pathogenicity to strawberry plants.

RELATIVE TOLERANCE OF DIFFERENT STRAWBERRY VARIETIES TO SUMMER DWARF

The following experiment was conducted at the North Carolina Coastal Plain Station, Willard, N. C. A plot consisting of 20 plants each of 36 different varieties of cultivated strawberries was established. The plants were secured from a dealer in Maryland and were transplanted on March 24, 1937. They were inoculated with culture-reared specimens of the southern strawberry strain of *Aphelenchoides fragariae* on June 23, 1937. The specimens were washed from the cultures with water, and concentrated in a small

amount of fluid by centrifuging. Three or 4 drops of this fluid were placed in the crown of each plant. Apparently, every plant had an equal opportunity to become infested. The nematode population was the same as that used in the experiments already discussed and had been maintained on culture continuously for 21 months. The data given in table 3 were secured on August 7, 1937.

TABLE 3.—*Development of symptoms of summer dwarf in different varieties of cultivated strawberries. Based on 20 plants of each variety*

Variety	Unquestionable symptoms	Possible symptoms	Dead
Aberdeen	6	4	6
Aroma	4	4	12
Beauty	11	3	6
Bellmar	7	2	4
Big Joe	8	–	9
Big Late	5	3	12
Blakemore	18	–	1
Catskill	8	–	7
Champion (= Progressive)[a]	5	–	11
Chesapeake	5	–	14
Clermont	9	–	9
Culver	1	3	3
Dorsett	16	2	2
Dr. Burrell (= Senator Dunlap)	7	–	11
Fairfax	13	3	3
Gandy	7	1	12
Gem[a]	1	–	17
Gibson (= Parson's Beauty)	8	2	9
Glen Mary	11	–	9
Green Mountain[a]	3	3	10
Haverland	2	–	18
Howard 17 (= Premier)	15	2	1
Klondike	12	1	5
Lupton	9	–	11
Marshall	6	2	10
Mastodon[a]	12	2	3
Missionary	14	1	3
Narcissa	12	–	4
New York	17	–	1
Orem	7	2	11
Pearl	6	1	16
Parson's Beauty (= Gibson)	5	–	13
Premier (= Howard 17)	11	1	4
Progressive (= Champion)[a]	1	3	15
Redheart	1	3	14
Sample	7	–	13
Senator Dunlap (= Dr. Burrell)	6	–	13
Southland	1	3	9
Wayzata or Rockhill[a]	7	–	11
Wm. Belt	8	3	5

[a] "Everbearing" varieties.

Some of the varieties of strawberries used in the experiment are known by more than one name. In several instances 2 bundles of plants were received from the grower, each under a different name, although, strictly speaking, both were the same variety. These were set in the plot under separate label.

Many of the varieties tested are not adapted to the climatic conditions of North Carolina. When final observations were made, November 7, 1937, the surviving plants of the varieties Haverland, Gem, Progressive, Redheart, Aroma, Green Mountain, and Wayzata were very small and weak. Lack of vigor was noticeable also in the varieties Chesapeake, Senator Dunlap, Parson's Beauty, Sample, and, to a less extent, in others. Failure of the plants of some of these varieties to survive and the poor growth of those that did survive is largely attributable to unfavorable climatic conditions.

The experiment demonstrated that all the varieties tested may become infested with the southern strawberry strain of *Aphelenchoides fragariae* and, when infested, may develop symptoms. The relative severity of symptoms varied considerably with different varieties. Glen Mary was the most severely affected, all the surviving plants showing exceedingly pronounced symptoms. Blakemore, Chesapeake, and Lupton also developed especially severe symptoms. Other varieties that developed severe symptoms, but perhaps not quite so severe as those already mentioned, were Bellmar, Catskill, Clermont, Dorsett, Fairfax, Klondike, Missionary, Narcissa, New York, and Premier.

Of the varieties on which symptoms were slight, the most outstanding was Beauty. Fourteen plants of this variety were obviously affected but all made a relatively vigorous growth and were not dwarfed, the abnormality consisting of distorted and crinkled leaves. Aberdeen, Culver, and Southland were other varieties on which symptoms did not appear to be very severe, but with these, especially the last two mentioned, failure to become infested may be the explanation. Symptoms on Mastodon were only moderately severe.

On the other varieties not mentioned above symptoms varied somewhat but, in general, seemed moderately severe. In some instances, as already noted, so few plants survived or those that did survive made such a weak growth that a reliable appraisal of the severity of symptoms was difficult.

SUMMARY AND CONCLUSIONS

When strawberry plants were inoculated with specimens of the southern strawberry strain of *Aphelenchoides fragariae* from agar cultures that had been maintained continuously for from 8 to 21 months, typical symptoms of summer dwarf usually resulted, both in the greenhouse at Washington, D. C., and in the open field at Willard, N. C. It is concluded that one may use culture-reared specimens of this nematode in experimental work with every assurance of securing the same results as would be secured were the nematodes obtained directly from the plants.

A plot composed of 20 plants each of 38 different varieties of cultivated strawberries was established at Willard, N. C. When inoculated with culture-reared specimens of the southern strawberry strain of *A. fragariae*, one or more plants of every variety developed typical symptoms of summer dwarf, but the severity of the symptoms varied considerably in different varieties. This is probably a matter of tolerance, and does not appear to be entirely correlated with vigor of growth.

SUGGESTED RÔLE OF ALKALOIDS IN PLANTS RESISTANT TO PHYMATOTRICHUM OMNIVORUM

GLENN A. GREATHOUSE

(Accepted for publication, April 9, 1938)

Taubenhaus and Ezekiel[1] have reported observations on more than 2,000 species or varieties of plants with regard to their relative resistance to *Phymatotrichum omnivorum* (Shear) Duggar. Of these approximately 19 per cent were found to be immune from the disease. A great part of the remainder were quite susceptible, although some species were resistant during most or part of their development.

Preliminary studies indicated the presence of alkaloids in crude extracts from roots of several plants resistant to *P. omnivorum*. A number of these complex substances have been isolated and shown to inhibit the growth of the fungus in the same or even lower concentration than that found in the plant tissue. To test further the possible rôle of alkaloids as inhibitors of the growth of the above-named organism, a survey of the literature was made for the purpose of noting possible correlations between the presence or absence of alkaloids and the classification of plants for resistance to *P. omnivorum*. Alkaloid-containing plants that have not been tested or observed for reaction to the root-rot organism were not included, although a considerable number of them are closely related to plants or groups known to be resistant or immune. The results of this study are set forth in table 1.

TABLE 1.—*Grouping of varieties and species of plants according to alkaloid content and their resistance or susceptibility to Phymatotrichum omnivorum*

Rating of plants as to disease reaction	Total	Extremely susceptible	Highly susceptible	Moderately susceptible	Resistant	Immune
	2116	54	375	876	403	408
Species in which alkaloids have been reported[a]	125	0	2	40	44	39
As percentage of the different groups			0.55	4.56	10.92	9.56

[a] Compiled from numerous publications on alkaloids.

Table 1 indicates a direct relationship between the presence of alkaloids in plants and their relative resistance to the Phymatotrichum root-rot organism. The percentages of the different groups are 0, 0.55, 4.56, 10.92, and 9.56, respectively, for the extremely susceptible to the immune group. It can be noted that 83 of the 125 plants that contain alkaloids fall within the resistant and immune groups.

[1] Taubenhaus, J. J. and W. N. Ezekiel. A rating of plants with reference to their relative resistance or susceptibility of Phymatotrichum root-rot. Texas Agricultural Experiment Station Bulletin 527. 1936.

Salgues[2] found that *Colchicum autumnale* bulbs, attacked by *Urocyctis (Tuburcinia) colchici*, contained only 0.044 per cent colchicine as against 0.115 per cent in those of healthy plants.

Schmidt,[3] and Agerberg *et al.*,[4] have shown that solanine caused abnormal type of growth in *Cladosporium fulvum*.

Nobécourt[5] suggested that alkaloids may protect plants against the attack of parasitic fungi. To test this hypothesis *Botrytis cinerea* was grown in Raulins solution to which different amounts of alkaloids were added. He studied the effect of only a few alkaloids and concluded that only one of the alkaloids used might occur in a plant in sufficient concentration to serve as a protection against this fungus.

The above studies, although of limited scope, and involving other diseases, tend to support the correlation indicated in table 1, and lend further support to the writer's proposal that the presence of alkaloids may partially explain the resistance of certain plants to Phymatotrichum root rot. It is not suggested that the mere presence of an alkaloid in a host plant necessarily warrants the conclusion that it plays a rôle in the resistance of that host to a given parasite or parasites, since recent work in our laboratory has shown that alkaloids with different nuclei or arrangement within a nucleus possess different inhibitory values. Furthermore, alkaloids may be present in concentrations so low that their inhibitory effects are negligible, or even stimulative to the organism.

Frequently more than one alkaloid is present in a given tissue. Therefore it is not essential that every alkaloid in the tissue be equally inhibitive in order to ward off or restrict the attack of the pathogenic organism. This fact has been confirmed by laboratory studies.

The relationship between the presence of alkaloids in the tissues of plants and the resistance of these plants to *P. omnivorum* is receiving intensive study. Meanwhile it was thought that this note might be of interest to other students of disease resistance since the literature on this subject is limited.

BUREAU OF PLANT INDUSTRY, IN COOPERATION
WITH TEXAS AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS.

[2] Salgues, René. Les modifications biochimiques en phytopathologie. Rév. Gén. des Sci. Pures et Appl. **48**: 237–244. 1937.

[3] Schmidt, Martin. Zur Entwicklungsphysiologie von *Cladosporium fulvum* and über die Widerstandsfähigkeit von *Solanum racemigerum* gegen diesen Parasiten. Planta, Archiv f. Wissenschaftl. Bot. **20**: 407–439. 1933.

[4] Agerberg, L. S., Rudolf Schick, Martin Schmidt and R. von Sengbusch. Die Bestimmung des Solaningehaltes von Pflanzen mit Hilfe von *Cladosporium fulvum*. Der Züchter **5**: 272–280. 1933.

[5] Nobécourt, Pierre. Physiologie végétale.—Action de quelques alcaloïdes sur le *Botrytis cinerea* Pers. Comptes Rendus **172**: 706–708. 1921.

SUSCEPTIBILITY OF CROP PLANTS AND WEEDS TO SCLEROTIUM ROLFSII

J. S. COOLEY

(Accepted for publication April 23, 1938)

Southern blight, caused by *Sclerotium rolfsii*, has been known since 1922 as a disease of apple stocks in South Africa.[1] It was reported as a nursery disease of apple trees in Australia in 1933,[2] and in 1936,[3] as a nursery disease of apple trees in this country.

In August, 1935, *Sclerotium rolfsii* Sacc., was observed in an apple-tree nursery at the U. S. Horticultural Field Station, Beltsville, Maryland. Again, in 1936, the fungus was in evidence and more trees, then 2 years old, were killed by it. After the trees were removed from the nursery in the spring of 1937, a plot where 20 per cent of the apple trees were killed in 1935 and 1936 was planted with 12 vegetable and forage crops to determine their susceptibility to the disease. The rows were run at right angles to those of the nursery in the previous planting.

The plants grown in this plot were pepper, tomato, eggplant, cowpeas, soy beans, navy beans, Kidney beans, red clover, Mammoth clover, alsike clover, spring oats, and Korean lespedeza, *Lespedeza stipulacea* Maxim. The seed was planted or the plants set on May 18. After about 3 weeks, 1 of 30 pepper plants, 1 Kidney bean and 2 cowpea plants out of several hundred were dead from the disease. After 4 months, 2 navy bean and 2 more cowpea plants were dead, but none of the clovers. On July 29, or 8½ weeks after planting, there was no further advance of the disease on the beans or peppers, but the lespedeza was severely affected. The disease seemed to have started at one or two points and advanced along the row till about 20 per cent of the plants were affected. On August 20, or 13½ weeks after planting, additional Korean lespedeza plants were killed, but there was no more advance on the other kinds of plants. By the end of the season, all the lespedeza plants were killed except those in a 6-foot section of a 30-foot row.

During the wet weather the latter part of the summer, most of the clover plants died, but the sclerotium disease was not apparent on them. This malady has been reported attacking clover, but, under these conditions, these 3 varieties apparently were unaffected. The remainder of the block, in which the disease was present in 1936 and was not used for this experiment, was planted to Wilson soy beans. They showed no apparent indication of injury because of the disease.

In July, cultivation was discontinued and weeds and wild grasses grew

[1] Birmingham, W. A. Sclerotium disease of Northern Spy stocks. Jour. Dept. Agr., Union South Africa 4: 405. 1922.

[2] Birmingham, W. A. Another fungus attacking apple stocks. Agr. Gaz., N. S. W., 44: 58–60. 1933.

[3] Cooley, J. S. *Sclerotium rolfsii* as a disease of nursery apple trees. Phytopath. 26: 1081–1083. 1936.

in the plot. In August, the following plants were noted as unaffected by the disease, even when growing in close proximity to diseased lespedeza: Pigweed, *Amaranthus retroflexus* L.; 3-seeded mercury, *Acalypha virginica* L.; Cuphea, *Cuphea petiolata* L.; ragweed, *Ambrosia artemisiifolia* L.; and foxtail grass, *Setaria glauca* L. Plants showing slight infection were spurge, *Euphorbia preslii* Guss. and common purslane, *Portulaca oleracea* L.; but the advance was not rapid nor was a large percentage of the plants affected. Since healthy plants, even of these two partially susceptible species, were growing about diseased lespedeza plants, it seems likely that, under these conditions, none of the weeds reported above are very susceptible to *Sclerotium rolfsii.* The fact that most of the other plants used, which are recorded in the literature as being susceptible to the disease in other regions, were so slightly affected under these conditions, may be because conditions were so unfavorable that only a few plants took the disease. Since this sclerotium disease heretofore has not been known to be serious as far north as this region, it seems very likely that climatic conditions are usually unfavorable here for its propagation and maintenance. Unfavorable conditions for the overwintering and infection of the disease in 1938 rather than lack of epidemic and abundance of sclerotia in 1937 probably account for the slight infection on plants that are reported in the literature as moderately susceptible. Even the highly susceptible lespedeza, which eventually was nearly all killed by the disease, showed initial infection at only 2 points in the row and spread from these foci of infection, killing all the plants as far as it spread. These observations point to the conclusion that, under these conditions, relatively few viable sclerotia survived the winter.

The results of this one year's experiment indicate that Korean lespedeza may be sufficiently susceptible to be useful as an indicator crop to determine whether or not a given plot contains the sclerotium disease. This experiment indicates that Korean lespedeza should not be used as a cover crop preparatory to planting apple nursery stock or other susceptible plants.

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Pythium irregulare and Damping off of Watermelons.[1]—Watermelon plants, whether inherently resistant or susceptible to *Fusarium bulbigenum* Cke. and Mass. var. *niveum* (E.F.S.) Wr., often damp off and wilt during the early seedling stage. This seedling loss has commonly been attributed to the wilt pathogen, but studies made recently indicate that certain other soil-inhabiting pathogens are partially responsible.

Fifteen isolations were made in the spring of 1937 at Conesville, Iowa, from field-grown, wilt-resistant, watermelon seedlings displaying wilted cotyledons, water-soaked hypocotyls, and, in some instances, parasitized roots. Thirteen of the fungi recovered were *Pythium* sp. One of the 2 remaining fungi was *Fusarium bulbigenum* var. *niveum,* and the other was an unidentified saprophyte. Eleven of the 13 *Pythium* isolates were tentatively assigned to *P. irregulare* Buisman, while the other 2 were not identified.

The pathogenicity of the different isolates of *Pythium irregulare* was demonstrated in 4 separate tests on artificially infested, autoclaved soil. In each of the 4 tests, 11 greenhouse flats contained infested soil were used, while 2 flats containing autoclaved soil were used as checks. Thirty surface-disinfected seeds of a wilt-resistant variety (Improved Stone Mountain No. 5) and a wilt-susceptible variety (Dixie Queen) were planted in each flat. The planted flats were held in a greenhouse where the temperature remained near 20° C. It was found that *P. irregulare* caused seed decay, preemergence and postemergence damping off, and root necrosis of the seedlings, regardless of their resistance or susceptibility to the wilt pathogen. The average stand of seedlings of the wilt-resistant variety, in artificially infested soil, was 37.3 per cent (30 days after planting), while that of the wilt-susceptible variety was 40.3 per cent. Average seedling stands of the 2 varieties, when planted in autoclaved soil, were 93.8 and 97.2 per cent, respectively. The pathogen was isolated repeatedly from infected roots, hypocotyls, and cotyledons of plants grown in infested soil, and also from seeds that had failed to germinate in such soil.

Fusarium bulbigenum var. *niveum* and *Pythium irregulare* produced similar damping-off symptoms under controlled conditions. The pathogens were capable of attacking roots, hypocotyls, and cotyledons of seedlings and of causing general necrosis. It is believed that seedling losses under field conditions are not entirely attributable to *F. bulbigenum* var. *niveum,* but that *P. irregulare* may be responsible for a portion of the damage.—S. G. YOUNKIN, Iowa Agricultural Experiment Station, Ames, Iowa.

[1] Journal Paper No. J-552 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 71.

Where Are Gardenia Cankers Initiated?[1]—Several investigators[2, 3, 4, 5, 6, 7] have found that *Phomopsis gardeniae* Hansen and Barrett, the cause of gardenia canker and gall, is a weak parasite. They have emphasized that the fungus enters the host through wounds made either by insects or during cultivation.

FIG. 1. Twelve week-old rooted *Gardenia florida* var. *veitchii* cuttings. Left: cankers at three nodes where leaves had been removed prior to embedding the cuttings in the propagating bench. Right: a more extensive canker.

[1] Journal series paper.

[2] Hansen, H. N., and C. E. Scott. A canker and gall disease of gardenia. Science (n.s.) **79**: 18. 1933.

[3] ———, and J. T. Barrett. Gardenia canker. Mycologia **30**: 15–19. 1938.

[4] Huber, G. A. A Phomopsis canker and gall disease of gardenia. Flor. Exch. and Hort. Tr. World **86**: 11. 1936.

[5] Obee, D. J. A note on the canker disease of gardenias. Trans. Kans. Acad. Sci. (1936) **39**: 103–104. 1937.

[6] Tilford, P. E. Stem canker disease of gardenia. Ohio Agr. Exp. Sta. Bimonth. Bull. **19**: 116–117. 1934.

[7] White, H. E. Careful culture recommended to avoid canker on gardenia. Flor. Rev. **79**: 9–10. 1937.

In continuing gardenia canker studies initiated by R. P. White at this station, it has been observed that a large percentage of infection starts at the leaf joints on the base of the cuttings after they are set in the rooting medium. The practice among commercial growers is to remove the lower leaves with a sharp knife before embedding the cuttings. These freshly cut surfaces furnish an excellent point of entrance for the fungus and are important foci for later infection.

Twelve-week-old rooted cuttings of the variety *veitchii,* with stem cankers, are shown in figure 1. The plant on the left shows 3 well-developed cankers located at the points where leaves were removed. Typical pycnidia and spores of *Phomopsis gardeniae* were visible in these cankers. Pure cultures of the fungus were obtained from each of the 3 cankers. The plant on the right shows a more extensive canker that apparently also started at the point where a leaf was removed.

This observation is in accord with that made by several commercial growers who have been unsuccessful in reducing the incidence of disease, even though they avoided unnecessary wounding of the plants during handling and cultivation, and kept the insect population at a minimum. Work is in progress to develop a wound dressing for protecting cuttings whose lower leaves are removed prior to being placed in the propagating bench.—P. P. PIRONE, Department of Plant Pathology, New Jersey Agricultural Experiment Station.

Chlamydospore production on artificial media by Urocystis gladioli.[1]—On February 8 of this year, H. W. Rankin, extension pathologist at this station, received for disease diagnosis some gladiolus bulbs from a grower at Fairview, Pennsylvania. This material was turned over to the writer for microscopic examination. Associated with the lesions were 2 or 3 *Penicillium*-like organisms and a smut, tentatively identified as *Urocystis gladioli* (Requien) Smith, until inoculation studies could be made to establish its true identity. This is believed to be the first report of the gladiolus smut from America.

Attempts were made to trace the origin of the smut, but our findings to date give us only the information that the bulbs had been grown in the above locality for the past 7 years.

Germination of the spore balls was undertaken by streaking them on potato-dextrose-agar plates. At the end of 12 hours germination was observed. In all observed cases it was akin to that of *Urocystis cepulae* Frost. (Fig. 1.)

In an attempt to free the smut spores from the ever-present *Penicillium,* dilution plates were made and individual spore balls were cut out of the agar and transferred to slants of potato dextrose agar on March 19. When these tubes were examined on March 27, several of them showed a *Penicillium* contamination, but 6 of them were free of this contaminant. These 6 tubes were alike in that the fungus present produced a reddish-brown prostrate

[1] Contribution No. 116 of the Botany Department.

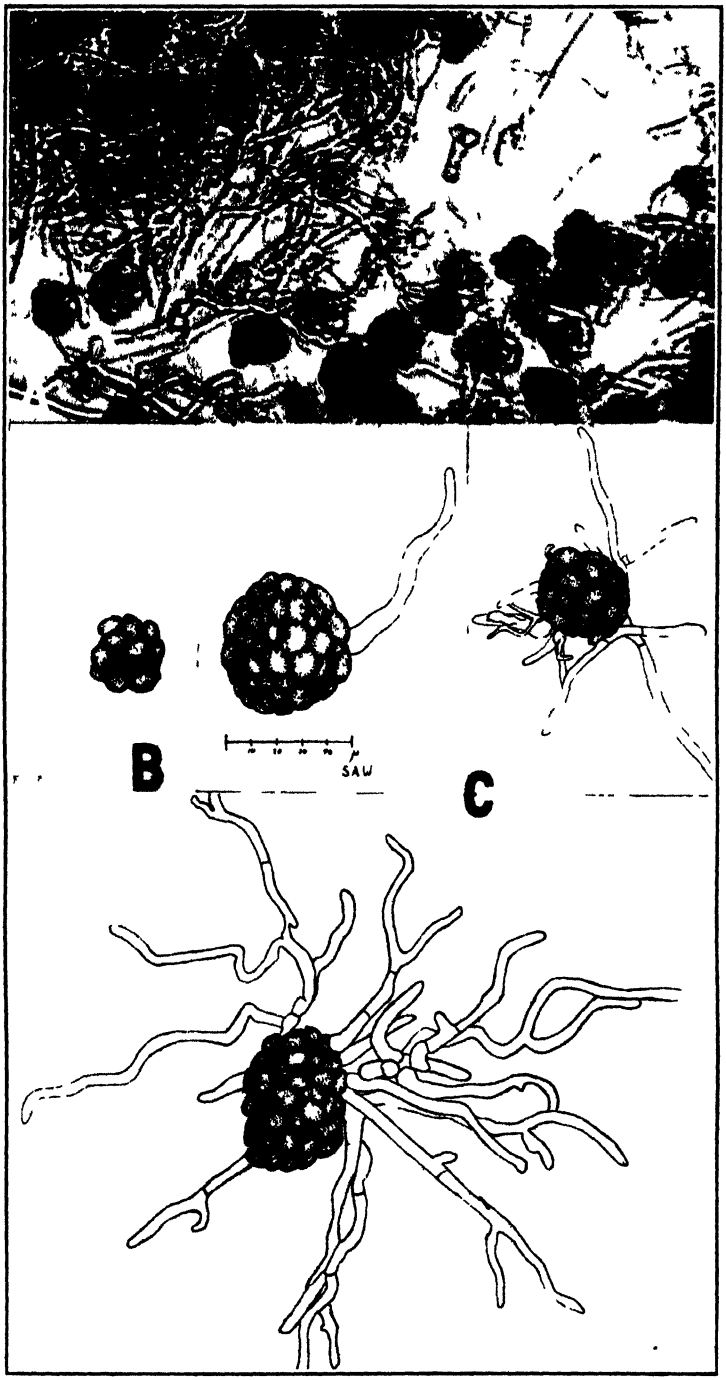

FIG. 1. A. Chlamydospores of *Urocystis gladioli* produced in a potato dextrose-agar culture. B and C. Chlamydospores from a gladiolus corm. C shows stages in germination.

mycelium of uniform appearance. Mounts were made from these tube cultures and examined under the microscope. Chlamydospores of the *Urocystis gladioli* type were present in large numbers in each mount. Subcultures of these spores have been made. The spores from these cultures germinate in the same manner as do those from the corms and at this writing are growing nicely.

This phenomenon concurs with that observed by Kneip[2] for *Urocystis anemones* (Pers.) Winter. To the writer's knowledge these are the only 2 species of *Urocystis* with which it has been shown possible to grow the smut from spore to spore under saprophytic conditions.

Acknowledgments are due to Professor L. O. Overholts for the photographs and to my wife for the drawings.—C. C. WERNHAM, Department of Botany, The Pennsylvania State College.

[2] Kneip, H. *Urocystis anemones* (Pers.) Winter. Zeitschr. f. Botanik. 13: 289–311. 1921.

NOTICE TO MEMBERS OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

At the Indianapolis meeting, the Society instructed a committee to formulate a list of technical words for which uniform international usage is highly desirable and to draft definitions for them for consideration at the Richmond meeting. It is planned that those words and definitions surviving the Richmond discussion be proposed at Stockholm for plenary action. Lists of words preferably with definitions, are solicited from all members of the Society. Attention is drawn to Wilbrink's list, Amsterdam Congress v, 2, p. 204. See also Crane, E. J., Words and Sentences in Science and Industry. Science 86: Dec. 17, 1937. Your committee are content to deal with a relatively small number of words but will welcome from any source a "complete" system. They feel that words of Latin derivation should be given preference wherever possible and they particularly solicit definitions in several languages. Communications should be addressed to the chairman and to be most useful should reach him before Sept. 1, 1938.

JESSIE I. WOOD
NEIL STEVENS
DONALD REDDICK, *Chairman.*

THE FORMATION OF COLORED ZONES BY WOOD-DESTROYING FUNGI IN CULTURE[1]

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(Accepted for publication May 5, 1938)

A number of investigators (1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 16, 20) have noted the presence of discolored zones[3] beneath the exposed surfaces of decayed wood that were subjected to drying. The discoloration in these cases was ascribed to the presence of masses of brown material in the wood elements. In most cases the observations were made on pieces of wood removed from decayed trees and allowed to dry in the laboratory. In a few cases (1, 4, 5, 10, 13), the studies were conducted with wood blocks that had been decayed by fungi growing in pure culture. Although the occurrence of discolored zones beneath the dry, exposed surface of decayed wood blocks has been observed frequently, no experimental work has been performed to determine the factors that dispose the mycelium to react in this manner. Various theories have been postulated, however, by observing the conditions that accompany the occurrence of the phenomenon. From such evidence certain workers (1, 2, 7, 12, 16, 21) have concluded that the zones consist of by-products resulting from the decomposition activities of the fungus; others (3, 4, 5, 6, 8, 13, 15, 20) have decided that the mycelium itself forms the brown masses that discolor the wood. The physiological causes of this type of zonation have been ascribed to the entrance of air into the wood (7, 11, 12, 16, 17), to desiccation of the mycelium (10), to unfavorable growing conditions for the fungus (3, 6, 20), to aging of the mycelium (13), and to deposition of toxic materials by the fungus (21).

There is considerable uncertainty as to the similarity of the discolored zones found in dried wood blocks and those associated with wood decay in

[1] This paper is a revision of a section of a thesis submitted in May, 1936, to the New York State College of Forestry, Syracuse, New York, as partial fulfillment of the requirements for the degree of Doctor of Philosophy. The investigation herein described was performed mostly in the Department of Forest Botany and Pathology under the supervision of Dr. Ray R. Hirt. Valuable suggestions were offered also by Dr. Henry F. A. Meier. A part of the work was done at the Botanisches Institut der Forstlichen Hochschule, Eberswalde, during 1932. To Dr. Johannes Liese, professor of the institute, thanks are due for active interest and advice.

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[3] The present confusion in terminology on this subject necessitates a delimitation of the terms used. Following is an attempt to select terms in conformity with present usage:

a) *Discolored zone:* An unnaturally colored region of the substratum the cause of which is associated with a pathological factor.

b) *Colored zone:* An organized group of pigmented hyphae located in the vegetative part of the hyaline mycelium. For this expression, Campbell (3) proposed and has subsequently used (4, 5) the term *pseudosclerotium*. It is considered advisable to exclude this term from the present discussion since the functional nature of the concept is inappropriate for describing the morphological aspect of the phenomenon.

c) *Zone line:* A discolored zone in the substratum that, when viewed macroscopically, appears as a narrow line. The use of the expressions *black line* and *black zone* for this term seems inadvisable since the color of the zones varies considerably under different conditions and with different fungi.

living trees. The possible relation between these phenomena emphasizes the necessity of obtaining a clearer understanding of the factors concerned in the formation of the discolored zones found in cultures. The determination of these factors was the object of the present investigation.

This study was performed by subjecting wood-destroying fungi in culture to various controlled environmental conditions. Since the discoloration referred to occurs in the form of restricted lines and narrow zones, it was apparent that those factors that vary quantitatively in different parts of the substratum probably induce the formation of zones. Light and heat conditions, which ordinarily are homogeneous within blocks decayed in culture, were, therefore, excluded *a priori* from consideration as the disposing factors in discolored-zone formation. Attention was given to other environmental factors, specifically to the effects of moisture and of the chemical composition of the atmosphere.

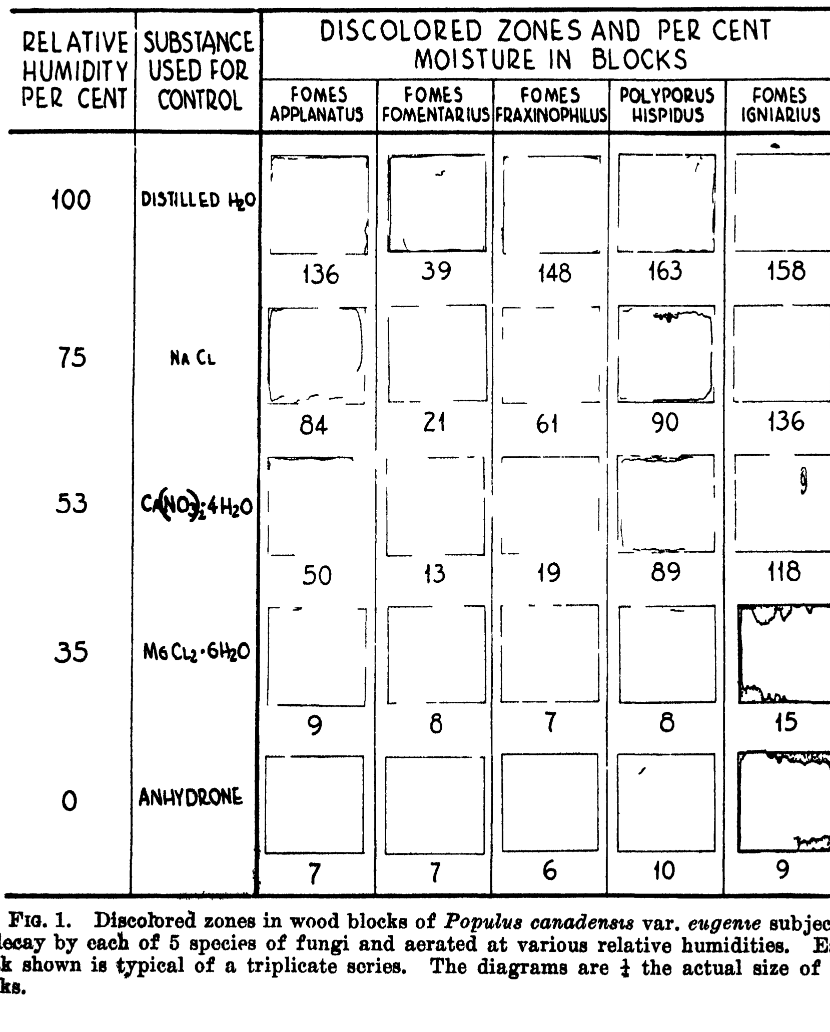

FIG. 1. Discolored zones in wood blocks of *Populus canadensis* var. *eugenie* subjected to decay by each of 5 species of fungi and aerated at various relative humidities. Each block shown is typical of a triplicate series. The diagrams are ½ the actual size of the blocks.

EFFECT OF HUMIDITY SURROUNDING DECAYING BLOCKS ON ZONE FORMATION

Since previous observations indicated that discolored zones formed when decayed wood was subjected to desiccation, an attempt was made to determine the effect of the relative atmospheric humidity on the process. This was accomplished by subjecting decaying wood blocks to different relative humidities in 5 sterilized chambers. The air in the chambers was constantly renewed, and its humidity controlled by saturated aqueous solutions according to a method previously described and illustrated (9, p. 29 and Fig. 3). The relative humidities within the 5 chambers and the substances employed to secure these conditions are stated in figure 1.

Cubical wood blocks of *Populus canadensis* var. *eugenie* Schelle, which measured 5 cm. on a side, were used as a substratum for the fungi. The blocks were partially evacuated of air by soaking them alternately in boiling and cold water until they sank. Each block was then placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker, and water was added almost to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton, which was held in place by the inverted half of a Petri dish. The culture blocks were then sterilized at 17 lbs. pressure for 30 minutes. After sterilization, small pieces of mycelium, removed from agar cultures, were placed on the blocks. The fungi on the wood blocks were then incubated in a saturated atmosphere, at 28° C., until the mycelium was established within the wood.

At the end of the incubation period (Table 1), some of the blocks were

TABLE 1.—*Pertinent data for experiment designed to test the relation between atmospheric humidity and the formation of discolored zones in wood blocks subjected to decay*

Fungus	Total number of blocks	Days of incubation in non-aerated vessels	Number of blocks examined at end of incubation period	Number of blocks with discolored zones at end of incubation period	Days the remaining 15 blocks were in the humidity chambers
F. applanatus	30	36	15	0	40
F. fomentarius	30	46	15	0	31
F. fraxinophilus	30	21	15	0	75
P. hispidus	30	37	15	0	39
F. igniarius	45	67	30	2	55

split open in order to determine whether discolored zones had formed. The remaining blocks were then subjected to controlled conditions in the humidity chambers, 3 blocks being placed in each chamber. After some days, as indicated in table 1, the blocks were removed from the humidity chambers and examined for the presence of discolored zones. The moisture content and specific gravity of a sample from each block also were determined. The following 5 species of fungi were used in the experiment: *Fomes applanatus* Pers., *F. fomentarius* (L.) Gill., *F. fraxinophilus* (Peck) Sacc., *Polyporus*

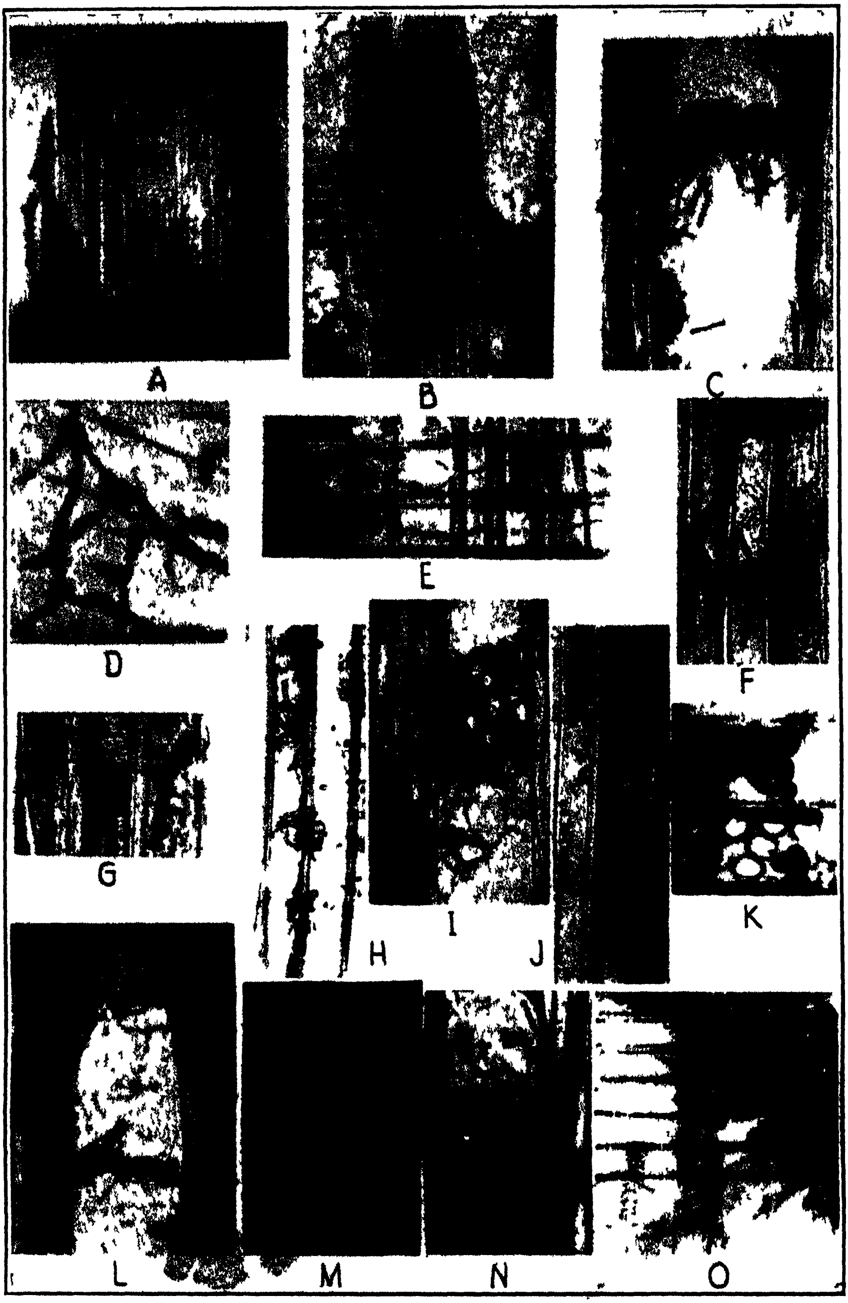

FIG. 2. Microscopic details of the colored zones formed in wood subjected to decay by various fungi in culture; A and B show wood of *Acer saccharum* Marsh., the remaining illustrations show wood of *Populus canadensis* var. *eugenie*. A, Zone line of *Fomes applanatus* beneath the exposed surface of a wood block, conterminous with the colored zone appearing in the mycelium on the outside of the block, $\times 6$; B, Zone line of *F. applanatus*, consisting of swollen, colored hyphae, $\times 135$; C, Zone line of *F. igniarius*, $\times 210$;

hispidus (Bull.) Fries, and *F. igniarius* (L.) Gill. These species were selected because discolored zones frequently are associated with their presence in nature.

RESULTS

During the course of the experimental period, several changes were noted in the color of the mycelium on the surface of the blocks. Significant observations in this regard are described below for each species of fungus. The distribution of discolored zones within typical blocks decayed by each species of fungus is illustrated in figure 1.

Fomes applanatus. At the end of the incubation period in nonaerated vessels, most of the surface mycelium on the wood blocks was hyaline. In a few cases, liver-brown (19) patches had appeared.[4] When placed in the aerated humidity chambers, the blocks that were exposed to 0 per cent relative humidity immediately dried out on the surface and, therefore, did not change in external appearance. At the higher humidities, the external mycelium on these blocks became liver-brown. The color of the surface mycelium at the end of the experimental period is given in table 2. Microscopic examination showed that the colored surface mycelium consisted of swollen, tan-color hyphae.

TABLE 2.—*Color of the mycelium of Fomes applanatus growing on the surface of wood blocks aerated at various relative humidities*

Percentage relative humidity	Color of surface mycelium: Number of blocks with hyaline mycelium	Color of surface mycelium: Number of blocks with liver-brown mycelium
0	2	1
35	1	2
53	0	3
75	0	3
100	0	3

[4] Other blocks that had been subjected to decay by this fungus were incubated at 100 per cent relative humidity in sealed vessels for 77 days. At the end of this period, the mycelium on the external surfaces of the blocks had become only slightly pigmented.

D, Irregularly expanded zone-line hyphae of *F. igniarius* in a wood vessel, ×460; E, Hyphae of *F. igniarius* in a wood ray, showing various aspects of the mycelium during the development of a zone line, including unpigmented, filamentous hyphae at the right of the illustration, irregularly expanded hyphae in the center of the illustration, and swollen, colored hyphae at the left of the illustration, ×330; F, Longitudinal penetration of the wall of a wood fiber by a colored, zone-line hypha of *F. igniarius*, ×350; G, Transverse penetration of wood fibers by a colored, zone-line hypha of *F. igniarius*, showing constriction of the hypha within the walls, ×360; H, Transverse penetration of the walls of wood fibers by zone-line hyphae of *F. igniarius*, ×225; I, Zone line of *F. fomentarius*, consisting of colored, expanded hyphae, ×125; J, Details of zone-line hyphae of *F. fomentarius* in wood fibers, showing expanded hyphae and amorphous, brown fungous excretions, ×185; K, Transverse penetration of the wall of a ray cell by the colored, expanded hyphae of *F. igniarius*, ×460; L, Zone-line mycelium of *Polyporus hispidus*, ×135; M, Zone-line hyphae of *F. fomentarius* penetrating the bordered pits of a wood vessel, ×250; N, Irregularly expanded zone-line hyphae of *F. fomentarius* in a wood vessel, ×150; O, Transverse penetration of the wall of a ray cell by the colored, expanded hyphae of *F. fomentarius*, ×207.

When the decaying blocks were split open at the termination of the experiment, zone-lines were found in several blocks. They were most prominent in those blocks that had been subjected to 75 per cent relative humidity. The lines were further below the exposed surface on the transverse faces, through which the external air could easily penetrate the wood, than on the longitudinal faces of the blocks. Where the lines extended to the external surface of the blocks, they formed the liver-brown patches previously referred to (Fig. 2, A). The hyphae constituting the colored zones appeared similar to those shown in figure 2, B.

Fomes fomentarius. During the period of incubation, profuse aerial mycelium grew over the surface of the blocks. This mat was removed when the blocks were placed in the humidity chambers. Subsequently, only a small amount of aerial mycelium formed. At 100 per cent relative humidity, a thin crust of brown mycelium formed over the surface of the blocks. A surface crust also occurred on portions of the blocks in 75 per cent relative humidity and on the top of 1 block in 53 per cent relative humidity.

Discolored zones were present inside 2 of the 3 blocks aerated at 100 per cent relative humidity. One of the blocks aerated at 53 per cent relative humidity also contained a light zone line. The zones, which were located just below the exposed surfaces of the blocks, consisted of swollen, colored hyphal masses (Fig. 2, I). The colored hyphae appeared similar to the mycelium constituting the surface crust.

Fomes fraxinophilus. Discolored zones occurred only in the blocks subjected to 100 per cent relative humidity. They were situated directly on, or just inside, the surface of the blocks. Microscopic observations showed that the zones consisted of masses of brown, swollen hyphae.

Polyporus hispidus. Discolored zones were present in some of the blocks in every chamber, but they were most prominent in the 3 blocks subjected to 75 per cent relative humidity. At 100 per cent relative humidity, discolored zones had formed in 2 of the 3 blocks. The 3 blocks exposed to 75 per cent relative humidity contained dark, prominent zones. At 53 per cent relative humidity, zones had formed in 2 of the 3 blocks. The blocks in 35 per cent and 0 per cent relative humidity contained a few, faintly-colored lines. The zones consisted of dark-brown, gnarled masses of hyphae (Fig. 2, L).

Fomes igniarius. Following several weeks of exposure to 100 per cent relative humidity in the fresh-air chambers, the surface mycelium became so profuse that the blocks were completely covered with a dense mass of brown hyphal filaments. The color of the mycelium was due to a brown pigment in the walls of the hyphae and to brown translucent droplets enclosed in the hyphal mass. The surface growth on the blocks placed in 75 per cent relative humidity was also profuse, but was only partly brown; most of the mycelium remained hyaline. Colored droplets exuded from the brown mycelium when it first formed, but after several weeks, the surface mycelium ceased growth and thereafter remained unchanged. The surface mycelium

on the blocks subjected to relative humidities of 53, 35, and 0 per cent dried out rapidly and remained hyaline. No new growth developed on these blocks after the first few days in the humidity chambers.

The occurrence of discolored zones in these blocks appeared inconsistent with the results obtained with the other species of fungi. The interiors of the blocks subjected to aeration at 100 and 75 per cent relative humidity were not discolored. Slight discoloration occurred inside only 1 of the 3 blocks exposed to 53 per cent relative humidity. Of the blocks placed in the chambers operated at 35 per cent relative humidity, 2 contained no zones, whereas the third block was prominently discolored. The wood just inside the exposed surface of the latter block was colored approximately cinnamon-buff (19). Farther into the block the wood was not discolored. A narrow zone line separated the discolored zone from the normally-colored portions. Microscopic observations showed that the colored line consisted of dark, thick-walled, gnarled hyphae (Fig. 2, C), present in the lumina of the wood elements. The discoloration of the wood between the zone line and the external surfaces of the block (Fig. 1) was due to the occasional presence of colored hyphae in the wood elements.

The interior of the 3 blocks subjected to 0 per cent relative humidity was discolored in a manner similar to that found in the block exposed to 35 per cent relative humidity. Several lighter colored, but otherwise typical, lines were found in the normally colored portion inside the discolored zones. The light color of these lines suggested that their maximum development had been inhibited, possibly by the rapid desiccation of the wood.

Although the humidity bore a definite relation to colored-zone formation by *Fomes igniarius*, the results were not in agreement with those obtained for the other fungi tested. On the basis of results thus far presented, this occurrence may be explained by either of 2 hypotheses. If specific percentages of relative humidity induced directly the process of colored-zone formation, the results of the experiment would indicate that *F. igniarius* reacted differently from the other fungi tested. On the other hand, if the effect of relative humidity were indirect, the results would indicate that the environmental complex that directly caused the formation of colored zones was distributed differently in the blocks decayed by *F. igniarius* than in the blocks decayed by the other fungi due to the physiological activity of the mycelium. The following 2 experiments were, therefore, undertaken in order to determine the effects of desiccation and aeration when applied directly to mycelium within the substratum. These tests indicated that the effect of relative humidity is indirect and that the second of the above hypotheses applied.

EFFECT OF HUMIDITY WITHIN DECAYING BLOCKS ON ZONE FORMATION

The effect of desiccation and aeration on the mycelium within blocks was determined by forcing a continuous stream of treated air under a slight pressure through decaying wood blocks by means of glass tubes that ex-

tended into the wood (Fig. 3). In this experiment, the mycelium within the wood was not subjected to various per cents of relative humidity. The object, rather, was to determine whether colored zones formed when hyphae in the wood elements were exposed (*a*) to aeration and desiccation, or (*b*) to aeration and moisture.

Cubical wood blocks of *Populus canadensis* var. *eugenie,* measuring 5 cm. on a side, were used. Two holes, 5 mm. in diameter and 25 mm. deep, were bored about 6 mm. apart on a radial surface of each block. Thick-walled, capillary, glass-tubing, 12 cm. long and 6 mm. in diameter, was forced into each hole. The blocks were then soaked alternately in hot and cold water

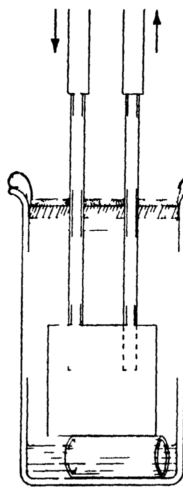

FIG. 3. Method used to aerate the hyphae growing within a wood block. × ⅓.

until they sank. Each block was placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker and water was added almost to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton. A circular piece of heavy cardboard, through which 2 holes had been bored in order to accommodate the glass tubes, was placed over the cotton and was forced down on the flanged lip of the beaker. Ten blocks were thus prepared; 2 served as controls, 4 were aerated at 100 per cent relative humidity, and the other 4 were aerated at 0 per cent relative humidity. The inlet tubes on the 4 vessels to be treated with desiccated air were joined with glass T-tubes, so that the air stream would enter the blocks at an equal rate. The outlet tube from each vessel extended into a vial of sterilized water. The ends of the tubes that were to serve as inlet and outlet for the air stream were plugged with absorbent cotton. The 4 vessels comprising the 100 per cent relative humidity set-up were treated similarly. The rubber tubing on the glass rods of the remaining 2 vessels, used as controls, was plugged with cotton and sealed with metal clamps. The blocks were then autoclaved at 17 lbs. pressure for 30 minutes.

After sterilization, a small piece of mycelium from an agar culture was

placed on each block. Two fungi were used, *Fomes applanatus* and *F. igniarius*. Five blocks were decayed by each species of fungus according to the following plan: 1 control block, 2 blocks aerated at 100 per cent relative humidity, and 2 aerated at 0 per cent relative humidity. Following establishment of the fungi on the blocks, the culture vessels were hermetically sealed. The rubber tubes were closed with metal clamps and sufficient melted paraffin was poured over the cardboard cover of each vessel to form a thick layer. The cultures were then incubated at 28° C. for 35 days in order to permit establishment of the mycelium within the blocks. At the end of the incubation period, aeration of the culture blocks was begun.

The apparatus used to aerate the cultures at 0 per cent relative humidity consisted of a desiccating tower containing Anhydrone, the outlet from which was attached to a sterilized filter tube packed with absorbent cotton. The air, which was desiccated and sterilized in this apparatus, entered 1 set of 4 culture vessels, being diverted into each vessel through the T-tubes. The humidifying apparatus, a description of which has been given in a previous paper (9, Fig. 5), consisted of a glass tower containing moist linen cloth. The air stream, which was sterile and saturated with water as it came from the apparatus, passed into the other set of 4 culture vessels.

RESULTS

After 42 days of aeration, the blocks were removed from the culture vessels and were split open. The presence of colored zones was noted in the case of each fungus (Fig. 4).

Fomes applanatus. Inside the control block, which had been subjected

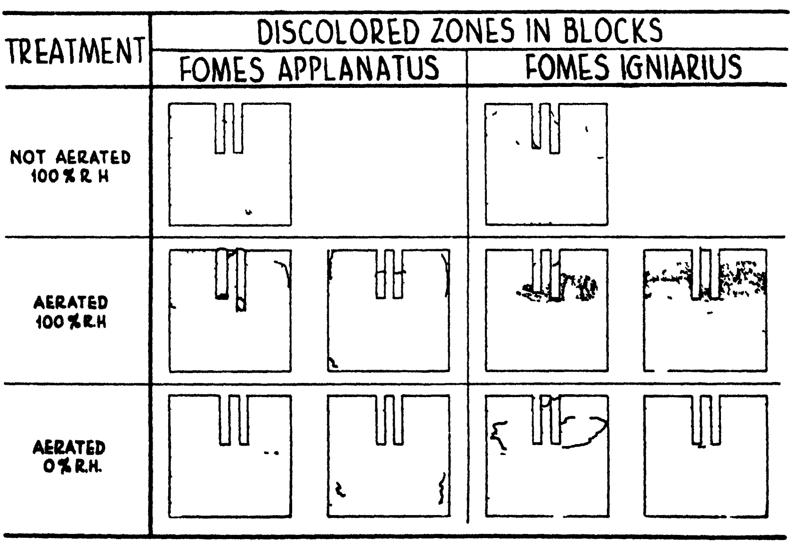

FIG. 4. Discolored zones inside decayed wood blocks of *Populus canadensis* var. *eugenie*, the interiors of which were aerated at 100 per cent and 0 per cent relative humidity. The dotted lines represent the longitudinal grain of the wood. The diagrams are ½ the actual size of the blocks.

to 100 per cent relative humidity but had not been aerated, 2 faint colored zones had formed. These were situated between the glass rods and the interior surface of the holes. In the 2 blocks aerated at 100 per cent relative humidity, prominent colored zones had formed. They were situated at the ends of the glass tubes in the wood, and in the portions of the wood where air from the holes could readily diffuse. The zones consisted of swollen, brown hyphae, similar to those shown in figure 2, B. In neither of the blocks aerated at 0 per cent relative humidity were any zones present in the vicinity of the holes. Zones were found, however, in the lower portion of 1 block. Their position in relation to the grain of the wood indicated that the dry air from the holes could not have diffused directly into the region where the lines occurred. In both blocks, the lower portions of the holes, which were not occupied by the glass tubes, contained a dense mass of hyaline mycelium.

Fomes igniarius. Colored-zone formation inside the control block was confined to a few, rather inconspicuous lines in the portion of the wood through which air from the holes could readily diffuse. In the blocks aerated at 100 per cent relative humidity, the wood around the tubes was markedly discolored. Several zone-lines were present in this region. The location of the discolored portion in relation to the grain of the wood indicated that the colored zones occurred in places directly exposed to the moist air stream. The discoloration was attributable to brown, gnarled masses of hyphae in the intracellular spaces of the wood. These hyphae were similar in appearance to those shown in figure 2, C. Only 1 of the blocks subjected to aeration at 0 per cent relative humidity had been dried out. In this piece, a few faint zones had formed. That the other block had not been completely dried out was evidenced externally by drops of condensed water, present on the inner walls of the culture vessel. This condition was caused by plugging of the glass tubes with a dense growth of mycelium. Although the drying effect on the outside of the block was slight, passage of dry air into the wood desiccated the region immediately around the glass tubes. This portion of the block was very dry and brittle, whereas the rest was wet. A prominent zone line demarked the boundary between the wet and dry portions of the block.

EFFECT OF HUMIDITY WITHIN DECAYING WOOD FLOUR ON ZONE FORMATION

The method of experimentation just described was applied in studying the effect of moisture and aeration on fungi growing in wood flour. The object in using wood flour was to permit observation of the mycelium during the process of colored-zone formation. This was accomplished by the procedure described below.

Round-neck bottles of 350-ml. capacity were used as culture vessels. Into each was placed about 200 ml. of wood flour, made from ground, air-dried wood of *Populus canadensis* var. *eugenie.* The material used was that that passed a 60-mesh standard sieve, but was retained by an 80-mesh sieve. Sufficient water was added to the culture vessels to saturate the wood flour. The vessels were stoppered with corks through which 3 holes had been bored. A

plug of absorbent cotton was placed in one of the holes. Glass tubes were extended through the remaining holes into the wood flour. The end of each tube was bent to a right angle, so that the opening of the tube just touched the inside wall of the bottle and was, therefore, visible externally. It was thus possible to observe the place where the conditioned air came in immediate contact with the wood flour.

Six culture vessels were thus prepared and were sterilized in an autoclave at 17 lbs. pressure for 30 minutes. Mycelium of *Fomes applanatus* was established on the wood flour in 3 of the bottles, and, in the 3 remaining bottles, *F. igniarius* was introduced. The mycelium was placed on the wood flour by momentarily removing the cotton plug from the hole that had been bored in the cork of each bottle. The corks were then sealed with paraffin in order that the only external supply of air to the mycelium was that entering the wood flour through the glass tubes. The 6 cultures were divided into 3 pairs, each of which consisted of 1 culture of *F. igniarius* and 1 of *F. applanatus.* The cultures were incubated for 42 days, during which time the ends of the glass tubes were sealed. At the end of this period, 2 pairs of cultures were subjected to aeration by forcing air into the wood flour through the glass tubes. Humidifying and desiccating apparatus similar to that used in the preceding experiment were employed. The remaining 2 bottles, which served as controls, remained sealed throughout the duration of the experiment.

RESULTS

After the air had been blown through the wood flour for 37 days, the experiment was terminated. Observations were made during this period in regard to the formation of colored zones by each of the fungi (Fig. 5).

Fomes applanatus. During the period of observation, no colored zones formed in the control culture.

After 19 days, the culture that was aerated at 100 per cent relative

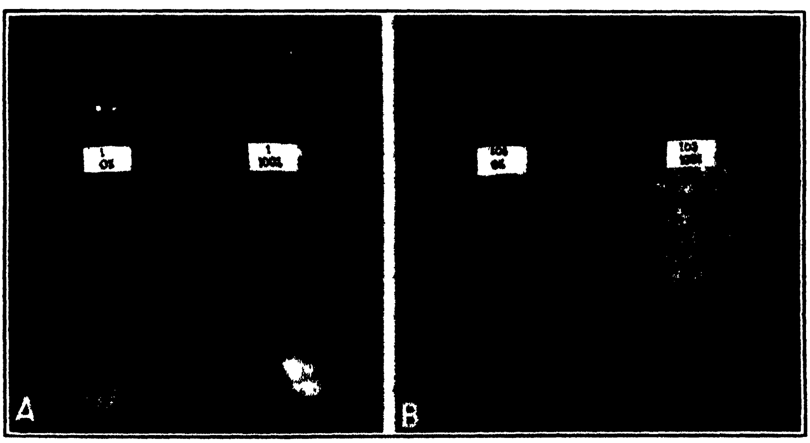

FIG. 5. Colored zones in wood-flour cultures which were aerated at 100 per cent and 0 per cent relative humidity. A, Cultures of *Fomes applanatus.* B, Cultures of *F. igniarius.* Control cultures (not shown in the illustration) did not discolor. × ⅓.

humidity contained a prolific growth of hyaline mycelium around the ends of the glass tubes. Some of these hyphae had become colored. The zones thus formed appeared on the inner wall of the bottle as dark lines. They consisted of brown, swollen, thick-walled hyphae of the type already described.

The wood flour in the culture aerated at 0 per cent relative humidity dried out without the formation of any zones. The wood surrounding the ends of the glass tubes became slightly discolored, but the typical colored zones, such as those present in the culture aerated at 100 per cent relative humidity, were not formed.

Fomes igniarius. No colored zones developed in the control culture during the period of the experiment.

In the culture aerated at 100 per cent relative humidity, prominent colored zones formed in the aerated region around the ends of the glass tubes. The zones appeared as irregularly distributed, dark lines consisting of masses of coarse, brown, thick-walled hyphae.

The wood flour in the culture aerated at 0 per cent relative humidity dried out rapidly. No colored zones formed.

EFFECT OF OXYGEN CONCENTRATION ON ZONE FORMATION

The previous experiments indicated that aeration of the mycelium was a controlling factor in colored-zone formation. An attempt was made, therefore, to determine whether the coloration was a response to the physical effect of air renewal or to the chemical action of the atmospheric gases.

The experiment was conducted by exposing mycelium to a reduced concentration of oxygen in an atmosphere saturated with moisture. The fungi were grown on wood flour in round-neck bottles, in a manner similar to that described in the previous experiment. Six bottles, connected together in pairs with T-tubes, were used. The wood flour in 1 bottle of each pair was decayed by *Fomes applanatus,* that in the other bottle by *F. igniarius.* The bottles were kept in an incubator at 28° C. for approximately 35 days. At the end of this time, the mycelium had become established throughout the wood flour in each bottle.

Following the period of incubation, saturated air containing oxygen of various concentrations was forced through the wood-flour cultures. One pair of cultures was exposed to air containing 20 per cent oxygen, 0 per cent carbon dioxide, and 80 per cent nitrogen. A second pair was treated with air containing 12 per cent oxygen, 8 per cent carbon dioxide, and 80 per cent nitrogen. The third pair was treated with oxygen-free air containing 2 per cent carbon dioxide and 98 per cent nitrogen. The cultures were observed for formation of colored zones.

RESULTS

Fomes applanatus

Twenty per cent oxygen. The culture was examined after 32 days of aeration. In this culture colored zones were present in the wood flour at the

ends of the glass tubes. The zones consisted of colored hyphae similar in appearance to those previously described for this fungus.

Twelve per cent oxygen. Colored zones formed also in this culture, but they were of a lighter color and were not so extensive as those that occurred in the culture exposed to air containing 20 per cent oxygen.

No oxygen. Air was blown through the wood flour for 38 days, but neither colored zones nor masses of hyaline mycelium developed. The appearance of such masses preceded the formation of colored zones in the other cultures.

Fomes igniarius

Twenty per cent oxygen. Prominent zones, consisting of colored hyphae similar to those previously described for this fungus, were present in the wood flour at the end of the 32-day period of aeration.

Twelve per cent oxygen. Several small zones formed in the wood flour. They were limited to the region in the immediate vicinity of the ends of the tubes.

No oxygen. No colored zones were noted in the wood flour after 38 days of aeration. The failure of this culture to form colored zones was not due to death of the mycelium, however, since subsequent aeration of the culture with air containing 12 per cent oxygen resulted in the formation of colored zones.

MORPHOLOGICAL NATURE OF COLORED ZONES

The typical morphological characteristics of the colored zones have been mentioned already. In the case of the 5 species of fungi studied, the zones consisted of swollen, gnarled hyphae showing various tones of brown. In some cases the hyphae were imbedded in a brown matrix. It was important to note that the microscopic appearance of the zones was characteristic for each species of fungus. The observation indicates the possibility of employing this character for the identification of vegetative mycelium growing in wood.

Formation of colored zones by *Fomes applanatus* began with expansion of the hyaline hyphae to a diameter of 20 to 30 μ. The swollen hyphae then became colored through the formation of a brown pigment. Colored material also was exuded into the lumina of the wood cells, the walls of which, however, did not become stained (Fig. 2, B).

Colored zones associated with *Fomes igniarius* formed in a somewhat similar manner. The zones began apparently with an increase in diameter and with pigmentation of the hyaline hyphae, portions of which then became enlarged to an average diameter of approximately 10 μ (Fig. 2, C, D, and E). The irregular expansions on the hyphae presented a gnarled appearance. At several of the places examined, the stout hyphae were colored but were not irregularly expanded. The pigment usually stained the cell walls of the wood and sometimes filled the lumina of the wood cells. Several peculiarities were noted in the form and location of the colored hyphae. In

places, colored hyphae had penetrated the pits of vessels, fibers, and ray cells (Fig. 2, H and K). Penetration of fibers by means of bore holes also was noted. According to Hubert (10, pp. 89 and 392), the bore holes produced by *F. igniarius* are very much larger than the hyphae. In the present study, this condition was usual throughout most of the decayed portion. The colored hyphae, however, usually were constricted where they had passed through the cell walls (Fig. 2, G). This observation indicates that in *F. igniarius* the type of cell-wall penetration is not constant. Another peculiarity was the longitudinal penetration of the middle lamella (Fig. 2, F), noted in both ray cells and fibers. Hubert (10) mentions only transverse penetration of the cell wall.

The hyphae constituting the colored zones of *Fomes fomentarius* (Fig. 2, I) developed from the hyaline hyphae apparently in a similar manner, that is, through swelling and pigmentation (Fig. 2, J and N). The swollen hyphae were about 20 μ in diameter, somewhat less than those of *F. applanatus.* Penetration of cell walls and pits by the zone-line hyphae was also observed (Fig. 2, M and O).

Formation of colored zones by other species was not studied in detail. Superficial examination of prepared slides indicated, however, that the zones of all the species studied had the same mode of formation. The zones of *Polyporus hispidus* (Fig. 2, L) and of *F. fraxinophilus* appeared somewhat similar to those of *F. igniarius.* The irregularly swollen hyphae that comprised the zones formed by these 3 fungi were approximately 10 μ in diameter, considerably less than that of the expanded hyphae of *F. applanatus* and *F. fomentarius.* The color of the zones differed with various fungi, being darkest in *F. igniarius* and *P. hispidus,* and lightest in *F. fraxinophilus.* The walls of the wood cells were discolored by *F. igniarius* and *P. hispidus*, but not by the other species studied.

DISCUSSION AND CONCLUSIONS

The results of these experiments showed for the 5 species of fungi tested that the occurrence of zones and lines of discoloration associated with wood decay was attributable to the formation of colored zones by the mycelium. The position of the colored zones, beneath and parallel to the exposed surfaces of the wood, indicated that the process of their formation was related to the external atmospheric conditions. Of these, aeration was apparently important, since coloration of the mycelium appeared in the blocks exposed within the aerated chambers, but did not generally form while the blocks were incubated in nonaerated vessels. Colored zones formed only when oxygen was contained in the air to which the mycelium was exposed. This indicated that coloration was a response to the chemical action of the atmospheric gases, not to the physical effect of gaseous renewal. The oxygen content of the atmosphere affected the prominence and probably the rate of colored-zone formation.

Likewise, the results definitely indicated that moisture relationships were

of significance in disposing the mycelium to form colored zones. The profuse growth and coloration of the surface mycelium on blocks aerated at 100 per cent relative humidity indicated that the presence of a saturated atmosphere was conducive to colored-zone formation. Since the zones within the blocks were confluent with and microscopically similar in appearance to the zones in the surface mycelium, it was to be expected that a high relative humidity would have the same disposing effect in their formation. The experimental results indicated this was apparently true for the fungi tested.

The effect of the external atmospheric humidity on the formation of colored zones within the blocks was apparently indirect in so far as it influenced evaporation of water and the consequent entrance of air into the wood elements. The proportion of air and water within the various blocks was approximated by moisture-content tests. The calculations indicated that colored zones did not occur in blocks that contained an extremely high concentration of water. This probably was because of the exclusion of the external air. The relation between the degree of saturation and the occurrence of colored zones was indicated particularly in the case of the blocks decayed by *Polyporus hispidus* at 100 per cent relative humidity. Two of the 3 blocks contained colored zones. These blocks had moisture contents of 163 and 167 per cent of the oven-dry weight. About 2/3 of the cellular space of the wood, therefore, was filled with water.[5] The third block, within which colored zones did not form, had a moisture content of 216 per cent of the oven-dry weight. Approximately 95 per cent of the cellular space contained water. It seemed probable that the concentration of water excluded air from the cellular spaces.

At humidities less than saturation, drying of the wood permitted external air to enter the cellular spaces and to come in contact with the mycelium. When the rate of desiccation was slow, as in the chamber operated at 75 per cent relative humidity, the mycelium in the cells of the wood was exposed to the external air and also was well supplied with water. It was under these conditions that the most pronounced formation of colored zones occurred. At the lower humidities, rapid drying permitted the entrance of air into the block, but free water was not long enough available to permit extensive zone formation. The necessity of interpreting the results in terms of the environment immediately surrounding the mycelium was noticed, particularly, in the case of the blocks decayed by *Fomes igniarius* in aerated chambers at various relative humidities. In this experiment, discoloration occurred only in blocks exposed to 35 and 0 per cents relative humidity. Since the

[5] The percentage of cellular space of the wood elements filled with water (W_f) was calculated by means of the following formula:

$$W_f = \frac{d \times sp.gr.(W_f - W_{f\,s\,p})}{d - sp.gr.}$$

in which d is the density of the cell-wall substance; *sp.gr.* is the specific gravity of the sample (oven-dry weight/wet volume); W_f is the moisture content of the fresh sample, expressed as percentage of the oven-dry weight; $W_{f\,s\,p}$ is the moisture content of the sample at fiber-saturation point, expressed as percentage of the oven-dry weight.

other tests had shown that the formation of zones by *F. igniarius* actually was inhibited by desiccation of the mycelium, it seemed apparent that at the time the zones formed in the wood blocks the mycelium was not dry. Moisture-content determinations on blocks exposed to relative humidities above 35 per cent indicated that the relatively great concentration of water in these pieces prevented aeration sufficient for colored-zone formation. In the blocks exposed to 35 and 0 per cents relative humidity, the rate of evaporation permitted entrance of air into the wood, but was probably too slow to cause rapid desiccation of the mycelium.[6]

Consideration of the results here progressively described led to the conclusion that colored zones formed only when mycelium was exposed concurrently to large quantities of air and water. There was, however, a certain degree of incompatibility in these conditions, since wood containing a large amount of free water was almost devoid of air. On the other hand, if the wood contained a large amount of air, it held only a small amount of intracellular water. Therefore, situations where mycelium was exposed simultaneously to large amounts of both air and water were very specific. For this reason, coloration was generally restricted in location to definite areas and appeared as narrow zone lines.

This conclusion tends to explain some of the apparently contradictory results obtained by other workers. Harder (6), working with *Xylaria*, noted that zones occurred on the surface of decayed blocks of wood exposed to moist air. He also noted that colored zones formed beneath the exposed surfaces, that is, in the outer cell layers of dried wood blocks that had been placed under water. Harder inferred that these 2 sets of conditions were so completely opposed that the direct disposing factor could not have been exposure of the mycelium either to air in the one case or to water in the other. He decided, therefore, that the formation of colored zones is a type of vitalistic phenomenon, which he termed *Dauerzustand*. When interpreted according to the results obtained in the present investigation, his observations indicate that the zones formed as a response of the mycelium to definite environmental influences. Harder noted that the formation of surface zones on wood exposed to air occurred only when the blocks were not dried rapidly. The zones formed under conditions that indicated the aerial mycelium was moist and well supplied with air. That surface mycelium colors under these conditions also was observed in the present investigation, as shown in the case of *Fomes applanatus* growing on blocks exposed to a high relative humidity (Table 2). The occurrence of zones inside dry pieces of

[6] Other, unpublished data indicate that under conditions comparable with those of the present experiment, *Fomes igniarius* liberates a considerable quantity of free water. This result may account for the relatively high concentration of water in the blocks aerated at 53 per cent and 75 per cent relative humidity, and offers an explanation for the possibility that aeration at a low relative humidity desiccated the mycelium inside the wood at a slow rate.

The physiological characteristics of the mycelia presumably account for the significant differences in moisture content of the blocks decayed by the different species. The water metabolism of the fungi, through its effect on the moisture content of the substratum, was, therefore, an indirect factor of importance in colored-zone formation.

wood that had been placed in water can be explained in a similar manner. The mycelium that formed the zones was exposed to air and water, only the relative position of these 2 agents was reversed from that existing in the pieces exposed to moist air. The supply of water to the mycelium was on the outside of the block and the air was contained within the wood elements. This situation was similar to that described in the present investigation in which the interior of moist blocks was aerated with saturated air.

Hubert (10) observed the occurrence of zones beneath the exposed transverse surfaces of large pieces of decaying wood placed in the warm atmosphere of the laboratory. He also noted the presence of zones within dried blocks decayed by various fungi in culture tubes. This worker attributed the formation of the zones to desiccation of the mycelium. As previously noted, however, it is important to distinguish between external drying of the blocks and desiccation of the hyphae within the blocks, when analyzing the effect of an indirect agent. The results of the present study indicate that when desiccation of the mycelium occurs, the formation of colored zones is inhibited. The effect of external drying is to cause evaporation of water from the wood, as a result of which the mycelium is aerated. Under these conditions, desiccation of the hyphae inside the wood may be only slight. A similar interpretation may explain the common observation, mentioned by Hubert, that discolored zones form "in the upper portion of rotted stumps in the region where excessive drying occurs, roughly parallel to the surfaces from which evaporation takes place."

Prillieux (16) noted the occurrence of a related phenomenon on the exposed transverse surfaces of logs decayed by certain fungi. The exposed end of a log decayed by *Polyporus hispidus* was protected from drying by placing the section upright on a piece of glass. After several days, brown mycelium grew from the region of advanced decay and formed a mat between the wood and the glass. Brown droplets of a liquid were exuded from the mycelium. The writer has observed a similar phenomenon when pieces of aspen and beech wood, decayed in association with the presence of *Fomes igniarius*, were placed on the laboratory floor. Brown mycelium grew over the protected end of the log, between the exposed surface and the floor. The brown, coarse hyphae that constituted the superficial mycelium extended a short distance into the decayed interior of the section. The hyphae appeared similar to those that constituted the colored zones formed by this fungus in culture. Brown mycelium did not grow from the other transverse surface, which was directly exposed to the dry external air, but typical zones were found in the wood beneath this exposed portion. Microscopic examination indicated that the formation of a mat of colored hyphae on the exposed surface of a decayed block is a process similar to the formation of zones within wood tissue exposed to dry air. According to this hypothesis, the sheet of glass on which Prillieux placed the wood section served to prevent desiccation of the wood surface, but did not exclude air from the mycelium. This condition disposed the fungus to color directly on the surface of the wood.

Nutman (13), working with *Polyporus hispidus,* concluded that only old hyphae formed colored zones; and he suggested that the process resulted from the utilization of all the available nutriment in the wood. Rudau (20) reached a similar conclusion from his studies on *Fomes igniarius.* That the occurrence of colored zones often is confined to old cultures, was confirmed in the present study. The age of the mycelium, however, did not induce the formation of zones; when mycelium was exposed to the proper environmental conditions, colored zones formed, even though the hyphae were relatively young. On the other hand, when the mycelium was rapidly dried out, or was not exposed to air, zones did not form, regardless of how old the hyphae became. The formation of zones in old wood-block cultures probably was ascribable to slow desiccation of the substratum. This permitted air to enter the cellular spaces and to come in contact with the mycelium. When the entrance of air into the blocks was experimentally induced, the formation of colored zones was hastened.

Baxter (1) found that colored zones were formed by *Fomes igniarius* and *Polyporus hispidus* when these fungi were grown on wood blocks for a year. This worker attributed the phenomenon to a deposition, in various portions of the wood, of fungous decomposition products. The present investigation indicated that the zones formed by *F. igniarius* and *P. hispidus* consisted, not of decomposed wood substances, but rather of dark-colored, coarse, thick-walled hyphae. Furthermore, the zones did not occur at indefinite locations but formed where the mycelium was exposed to specific environmental conditions. In Baxter's work, zones formed only when the fungus was growing on wood or on agar substrata containing wood. He, therefore, concluded that formation of the zones was related in some way to the wood itself. In the present study, zones were occasionally formed on nutrient-agar cultures containing no wood. It seemed, therefore, that the presence of wood was not a requirement for colored-zone formation.

The factors that induced the formation of zones in agar cultures were seemingly identical with those that induced the mycelium to form zones in wood. This was found by aerating the interior of malt-agar cultures in Erlenmyer flasks, in a manner similar to that used to aerate mycelium growing in wood flour. It was observed, however, that colored zones formed less frequently on nutrient agar in Petri dishes and in culture tubes than on wood substrata. Probably the physical condition of the agar gel prevented exposure of the mycelium within the medium to the external air. The mycelium growing on the surface of the agar, on the contrary, was exposed to a large quantity of air and also was well supplied with water. It was reasonable to expect that, under these conditions, the entire surface mat of mycelium would color. Actually, when the 5 species of fungi experimented with in this investigation were grown on malt agar, the surface mycelium became brown. The subsurface mycelium, on the other hand, remained hyaline. The colored surface hyphae, especially those of *Fomes applanatus,* appeared similar to the hyphae comprising the colored zones formed in wood. The morphological

similarity between these 2 types of colored mycelia, as well as the similarity in the accompanying conditions of moisture and aeration, suggested that the phenomena were closely related. Panisset (14), working with *Daldinia concentrica* (Bolt.) Ces. et de Not., observed a similar morphological relation between the surface mycelium growing on agar and the colored-zone mycelium in the wood.

SUMMARY

An attempt was made to determine the nature and origin of the discolored zones that occurred in association with decay of the wood of *Populus canadensis* var. *eugenie* in culture by 5 species of fungi: *Fomes applanatus, F. fomentarius, F. fraxinophilus, Polyporus hispidus,* and *F. igniarius.* Microscopic study of the discolored zones showed that the phenomenon was not attributable simply to deposition or production of a pigment in the wood, but was a vital process of the hyaline type of vegetative hyphae. In the material studied, the zones consisted essentially of swollen, gnarled hyphae, associated with a brown pigment secreted by the mycelium. These zones were typical in microscopic appearance for each of the fungi studied. It was found possible to induce coloration of the hyaline type of mycelium by experimentally controlling various environmental factors. The environmental complex that stimulated coloration was the concurrent exposure of hyphae to large concentrations of water and air. This reaction occurred only in the presence of atmospheric oxygen, although the process was not one of oxidation.

Within wood, large concentrations of water and air were present concurrently only in restricted layers; hence, the pigmentation usually appeared as discolored zones or dark lines. A more extensive but similar type of coloration developed in mycelium growing on the surfaces of agar and moist wood. The following factors, through their effect on the moisture and air distribution within the substratum, had an indirect influence on coloration: (*a*) physical structure of the substratum, and (*b*) relative humidity of the air. Since pigmentation of the mycelium is a natural phenomenon resulting from definite stimuli, the vital process has been herein referred to as colored-zone formation. Coloration of the mycelium results in the discoloration of the wood; restriction of the coloration accounts for the presence of zone-lines of decay in the wood substratum.

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SEED TRANSMISSION OF MACROPHOMINA PHASEOLI

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INTRODUCTION

The disease of beans, *Phaseolus* spp., caused by *Macrophomina phaseoli* (Maubl.) Ashby, in the United States, and commonly referred to as ashy stem blight, was, according to Kendrick (6), first observed by J. T. Barrett in California in 1919. Since there appears to have been no published record of this observation, the same disease was described in 1925 as "A new stem rot of beans" by Ludwig (7), in South Carolina, who identified the associated pycnidial fungus as *Macrophoma phaseoli* Maubl., later assigned to the genus *Macrophomina* (3).

At the time of its rediscovery in the United States, it was apprehended that the new disease might become a serious menace to the bean-growing industry. Publicity was given to it in the Science News Supplements (1) and in at least one trade journal (2). The disease was discussed by Ludwig at the Plant Disease Survey Round Table in Philadelphia in 1926. At that time he proposed the name "ashy stem blight." From year to year for the past several years since its first discovery in South Carolina, brief reports on the occurrence of the *Macrophomina* on beans, Lima beans, and cowpeas, chiefly in the southeastern States, have been filed in the records of the Divi-

sion of Mycology and Plant Disease Survey. Occasionally, local plantings would suffer severe losses induced by the fungus. In general, the reports indicated the disease to be of doubtful importance, although of wide distribution in the warmer bean-growing regions of the United States.

In 1932, Mackie (8) described damage caused by *Rhizoctonia bataticola* (Taub.) Butler in California as "a hitherto unreported disease of maize and beans." The *Rhizoctonia bataticola* and *Macrophomina phaseoli* are now commonly understood to be growth forms of the same species (5).

Kendrick (6), in 1933, and Tompkins and Gardner (9), in 1935, testify that *Rhizoctonia bataticola*, unaccompanied by the pycnidial (*Macrophomina*) stage, is capable of causing serious losses in the hot, interior valleys of California where the largest commercial plantings of beans are found. These investigators have proved definitely that pure cultures of *R. bataticola* are able to parasitize young bean plants under particular environmental conditions, the most important one of which appears to be high soil temperature.

The writer has proved that pure cultures of *Macrophomina* (with pycnidia) will, likewise, parasitize young bean plants, with symptoms distinguishable from those attributable to *Rhizoctonia* only by the presence or absence of pycnidia in advanced lesions. Data bearing upon this point, however, will be referred to sparingly in this paper. The data presented here were obtained with a quantity of southern-grown Henderson Bush Lima bean seed that proved to be infected by *Macrophomina phaseoli*.

METHODS

Sterilized (autoclaved) soil in flats was used in all greenhouse plantings and sterilized (3 minutes in HgCl, 1–1000) and unsterilized seed were planted, usually in parallel lots. The experiments extended through July, August, and early September, with daytime air temperatures usually 90° to 110° F. in the greenhouse. Temperatures fell to below 70° F. at night and ranged up to 125° F. for brief periods in full sunlight.

The greenhouse experiments were made to determine the effect of deep and shallow planting, slow and rapid germination, high and low temperatures, and high and low humidity on the course of infection, although in most cases these conditions were not under strict control.

Field plantings with nonsterilized seed were made on June 30, July 14, July 28, and August 11, using a total of 8,000 seeds in 8 12-rod rows in an overhead irrigation plot. From the standpoint of rainfall, the season was unusually favorable and only a single irrigation was made on July 10. The 2 first plantings were allowed to grow until killed by frost in October, and information was obtained on the later history of incipient stem lesions.

Formal proof that the seedling infections obtained were from infected seed consists of the following:

1. The fungus was observed on, developed on, and isolated from surface-sterilized seed.

2. The fungus developed on plants from surface-sterilized seed planted in sterilized soil.

3. No Macrophomina infections occurred on several thousand bean and Lima bean check plants grown in greenhouse and field during the same period.

Primary Infection from Diseased Seed

Kendrick (6), Tompkins and Gardner (9), and Dastur (4), in studies on beans, cowpeas, and cotton, all emphasize the importance of the cotyledons in primary infections by *Macrophomina phaseoli* (or *Rhizoctonia bataticola*). With the fungus established in the cotyledon, it seems unimportant whether the infection of the seed comes after it is planted or before it is harvested, since in all likelihood the infection of seed in the pod would occur through contact of the pod with infested soil. The occurrence of natural seed transmission, nevertheless, may be an important circumstance in accounting for the occasional serious outbreaks of the disease in regions where home-grown seed is apt to be used. Furthermore, in the writer's experiments, consistently more seedling infection is obtained when the seed is infected before planting than when infection is depended upon to occur from inoculum in the soil. The fungus in a pre-infected seed may have the advantage of no more than a day's growth; yet, even a few hours difference in the incubation period may affect the amount of damage caused to seedlings by the *Macrophomina*, which grows with great rapidity at high temperatures and on a favorable substrate.

The artificial infection of bean seed by pure cultures of *Macrophomina* has been accomplished repeatedly, and a higher percentage of seedling mortality is obtained from such pre-infected seed than from healthy seed planted in heavily infested soil.

The first direct evidence of natural seed infection by *Macrophomina* was obtained in September, 1935, when pycnidia of the fungus were found on a few mature pods in the experimental plots at Beltsville, Maryland. The pycnidia were numerous on the exterior of the pods and contained normal *Macrophomina* spores. The seeds also were obviously infected but bore no pycnidia. Isolations from the surface-sterilized seed coats and from the cotyledons all gave sclerotial cultures of *Macrophomina*.

During the summer of 1937 it was possible to demonstrate that seed transmission of *Macrophomina phaseoli* may be of economic importance. With the cooperation of W. D. Moore, about 10 lbs. of home-grown Henderson Bush Lima bean seed were obtained from a farmer near Tifton, Georgia, who had planted 20 acres with seed of the same source and had obtained only a 5 per cent stand.[1] The principal cause of the damage was identified by Dr. Moore as *M. phaseoli*, and specimens of diseased seedlings collected 12 to 16 days after planting bore symptoms resembling those described by Kendrick (6) and Tompkins and Gardner (9) on bean and cowpea seed-

[1] The writer wishes to express gratitude to Dr. Moore for his generous cooperation in this work.

lings, and identical also with those obtained by the writer on bean seedlings with pure cultures of *M. phaseoli*. Pycnidia were abundant on the blackened stems. As in all similar material, the lesions extended predominantly from the cotyledonary node.

Many of the seeds were visibly infected by *Macrophomina*. Numerous small, black sclerotia beneath the seed coat gave an ashy-grey appearance to the exterior of the seed. Laboratory germination tests were made on moist blotting paper, using seed, surface-sterilized 3 minutes with 1–1000 mercuric chloride. *Macrophomina* developed on 57½ per cent of the seed, or 212 out of 369. Only 31 per cent germinated, principally because of the rapid decay caused by *Macrophomina*. Pycnidia developed on most of the infected seed but isolations from these gave only sclerotial cultures of *Macrophomina*. Seed from the same lot was used in a series of greenhouse and field-planting experiments made at intervals of 1 to 3 weeks, most of the results of which are shown in tables 1 and 2.

The total amount and character of injury to plants developing from the infected seed varied greatly in plantings made at several different times and under different conditions. Table 1 shows that in 5 lots of 500 seeds each, planted at intervals of 1 to 3 weeks, there was a regular increase in percentage germination and a regular decline in the percentage of plants infected. These results do not correlate satisfactorily with greenhouse temperature records. If, however, comparison be reduced to the 2 extremes (July 1 and August 25), there seems to be correlation between temperature and infection. In figure 1, A, the 10-day infection period is divided into 2 phases, the preemergence phase and the postemergence phase. It is possible that temperature exerts an opposite effect in these 2 phases of the infection period; if so, the curves in figure 1, A, could be interpreted as follows: Higher temperature in the preemergence phase favors rapid embryo development and reduces seed decay, whereas higher temperature in the postemergence phase may favor the pathogen (with an optimum of *ca* 35° C.) more than the host.

In similar data from field tests (Table 2 and Figure 1, B) the lot with higher temperature in the germination period also gave the highest percentage germination and lowest percentage infection.

These data are somewhat contrary to the results of Kendrick (6) and Tompkins and Gardner (9), who found that high soil temperatures favored infection by the sclerotial form of the pathogen. The discrepancy may mean either that temperature is not the principal controlling factor in the disease or that the actual temperature level at Beltsville was too low to reveal the maximum effect of temperature on the pathogen. Data were not available on soil temperatures in the Beltsville experiments.

Data in table 1 show that surface-sterilizing the infected seed consistently reduced the percentage of plants infected by approximately one half. The nonsterilized series shows the same seasonal increase in percentage germination and decrease in percentage infection as occurred in the sterilized series.

TABLE 1.—*Greenhouse Series: Relation of date of planting and seed treatment to development of Macrophomina phaseoli on Henderson Bush Lima bean seedlings from naturally infected seed planted in the greenhouse in 1937*

Date of planting	Seed treatment	No. seed planted	No. plants emerged	Percentage emerged	No. of emerged plants infected	Percentage emerged plants infected	Percentage leaves infected
July 1 ...	Sterilized	500	297	59.4	88	29.6	....
July 21 ...	"	500	274	54.8	80	29.2	. . ..
Aug. 4 ...	"	500	313	62.6	69	21.8	.. ..
Aug. 25	"	500	355	71.0	41	11.5	... .
Aug. 18	Sterilized + high humidity	500	336	67.2	5	1.5	49.4
July 21 ..	Unsterilized	500	163	32.6	99	61.0	. ...
Aug. 4	"	500	288	57.6	172	59.7	....
Aug. 25	"	500	335	67.0	103	30.7	....
Aug. 25	"	500	347	69.4	109	31.4	....
July 21	Sterilized + deep planted	500	229	45.8	36	15.7	....
Aug. 18	Unsterilized + high humidity	500	338	67.6	30	8.8	59.1

TABLE 2.—*Field Series: Relation of temperature to development of Macrophomina phaseoli on Henderson Bush Lima bean seedlings from naturally infected seed planted in the field at Beltsville, Maryland, in 1937*

Date planted	Seed treatment	Seed planted	Av. daily mean temp. in °F. for 10 days after planting	Av. daily mean temp. of preemergence phase	Av. daily mean temp. of postemergence phase	No. of plants emerged	No. of emerged plants infected	Percentage germination	Percentage infection
June 30	None	2000	73.6	69.4	77.8	693	366	34.6	52.8
July 14	None	2000	81.5	82.8	80.2	1041	168	52.0	16.1
July 28	None	2000	74.1	74.4	73.8	711+[a]	228±	35.5+	32.0±
Aug. 11	None	2000			77.8	823	307	41.1	37.5

[a] Results of this test were complicated by worm infestation.

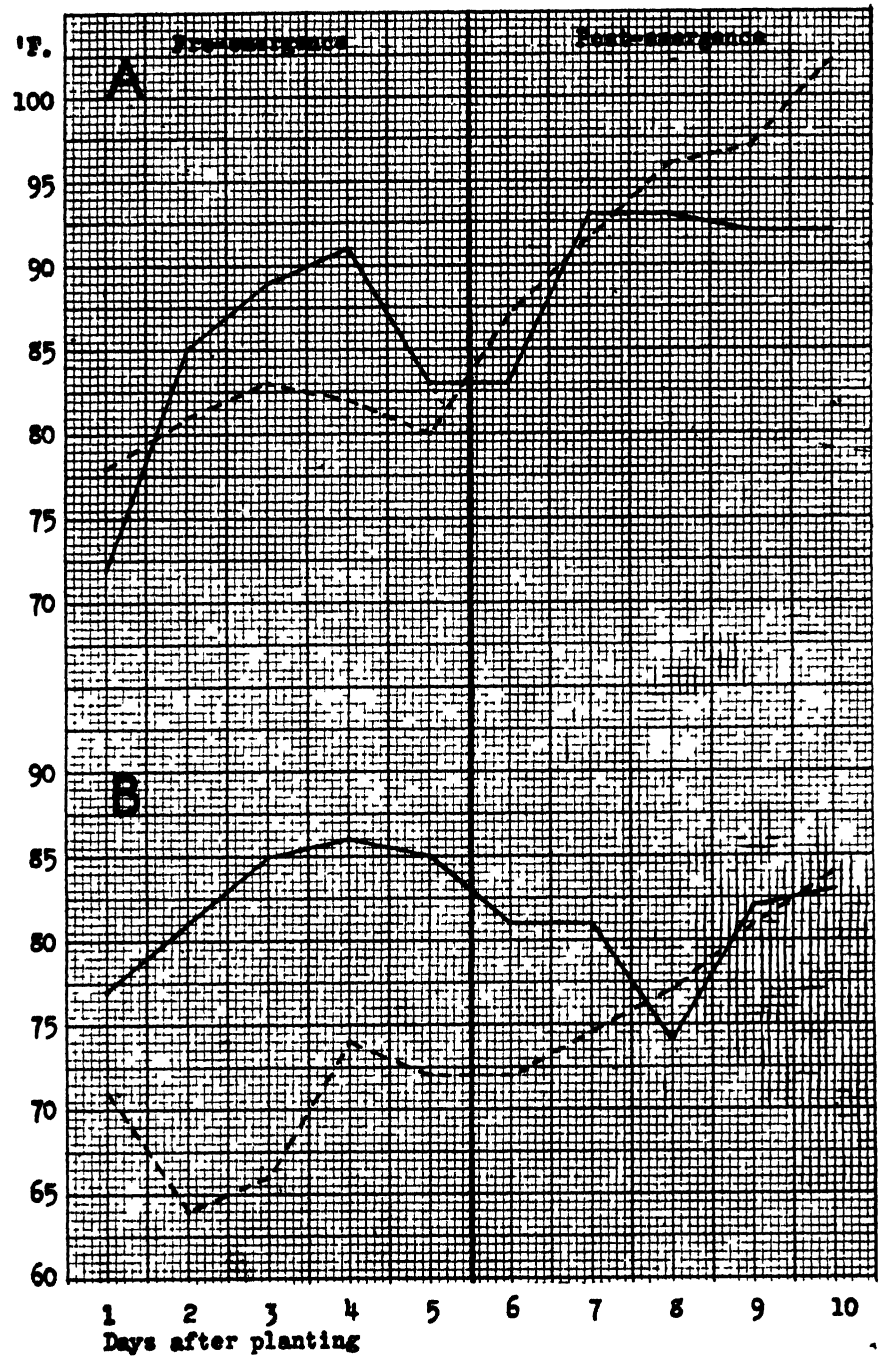

FIG. 1. Correlation between temperature and seed borne-Macrophomina infection. A. In greenhouse: broken line, low germination and high infection (July 1 to 10); solid line, high germination and low infection (Aug. 25 to Sept. 3). B. In the field: broken line, low germination and high infection (June 30 to July 9); solid line, high germination and low infection (July 14 to 23).

It might be supposed that the pathogen merely declined in viability in the seed were it not for the fact that field data do not show the same seasonal change.

The 2 lots planted August 18 (Table 1) show the remarkable effect of rapid germination and rapid emergence. These lots were kept in glass-covered infection chambers for 7 days after planting. Seedlings began to emerge on the second day after planting because of the higher temperature and especially favorable moisture conditions. Seedlings elongated rapidly and became extremely etiolated. On the 5th day, cloudy and cold weather set in. Very few infections occurred at the cotyledonary node, but a high percentage of primary leaves were infected as a result of contact with infected cotyledons accompanied by excess moisture. Pycnidia developed on necrotic portions of the infected leaves. When the plants were removed from the humidity chambers, development of the lesions ceased abruptly. When returned to the humidity chambers, the lesions resumed development and in several cases the infection progressed through the petiole into the stem and produced ashy-stem-blight symptoms.

The seedling phase of Macrophomina infection is definitely distinguishable from the disease on older plants. The term "ashy stem blight" originally referred to older plant infections where an "ashy" color was imparted to stems and petioles by the presence of numerous pycnidia underneath the translucent epidermal layer. The seedling phase is a type of damping off in which the term "charcoal rot," used by Tompkins and Gardner (9) and others, adequately describes the deep black lesions. Pycnidia never developed on the material used by Tompkins and Gardner, while, in the present case, they developed externally in advanced lesions and subepidermally only on older plants. The ashy appearance is thus absent in the seedling phase.

Typical infection occurs from the cotyledonary node (Fig. 2). Once established beneath the seed coat, the fungus is able to penetrate the stem through the base of the cotyledon. A few instances were observed where infection had begun at the tip of the plumule and on the main root, probably from contact with the infected seed coat during germination. In the former instances the primary leaf became completely blasted and the fungus proceeded through the petiole into the stem.

Apparently, some degree of infection of the seedling is essentially assured, provided the fungus becomes established in the seed coat before germination. Conditions favoring the adherence of the infected seed coat to the cotyledon probably will favor infection. Likewise, conditions favoring adherence of the cotyledons to the stem will favor stem invasion. Infected cotyledons frequently adhere to the stem long after the noninfected cotyledons have dropped off, due possibly to the presence of mycelium at the point where abscission would normally occur.

Death of the seedling seems to result when the fungus invades the stem before the seedling is completely emerged from the soil. If invasion of the stem is delayed beyond that stage, only an incipient lesion develops at the

FIG 2. Seed-borne *Macrophomina phaseoli* infection of Henderson Bush Lima in the greenhouse showing degrees of seedling injury 7 days after planting (above) and 20 days after planting (below). Approximately natural size Photographs by Guernsey.

cotyledonary node or the infected cotyledon is cast off before the fungus reaches the stem and no stem infection results. Apparently, any condition, either hereditary or environmental, that prolongs the germination period will favor seedling infection unless it has, at the same time, a sufficiently depressing effect upon the parasite.

Some results indicate that deep planting of infected seed increases the number of seedlings killed prior to emergence, but does not increase the total number of infected plants (Table 1). When noninfected seed is planted in inoculated soil, deep planting probably would increase the total percentage of infection because of the longer time required for the plants to emerge above the soil.

Tompkins and Gardner (9) considered the relative freedom from infection by *Rhizoctonia bataticola* of cowpeas as compared with beans at high temperatures as attributable in part to their more prompt germination and more rapid hypocotyl elongation. Also, Dastur (4), in reference to *Macrophomina phaseoli* infection of cotton seedlings, states, "Any delay in the appearance of the cotyledons above the soil surface favors infection of the seedling. Seeds that are naturally very slow in sprouting are particularly susceptible; and, where soil conditions themselves are unfavorable to quick germination, heavy losses may occur."

Under usual conditions of growth, the parasitism of the fungus on beans seems strictly limited to the seedling host. Progress of a lesion is stopped abruptly after the first few days growth of the plant. Thus, on many plants, incipient lesions are observed in the cotyledonary scar, where penetration of the stem by the fungus was for some reason delayed (Fig. 3). The incipient lesions ordinarily make no further growth during the lifetime of the plant, although they may be extensive enough to cause breaking at various stages before the plant reaches maturity. Growth of callus tissue within the dormant lesion sometimes obliterates evidence of seedling infection.

Secondary Infection

It has been pointed out that the primary infection of the seedling from infected seed ceases its development abruptly during the first few days after emergence of the seedling aboveground. Stem lesions may remain throughout the life of the plant without undergoing any appreciable further growth (Figs. 3 and 4). At the same time, it is well known that infections do occur on older plants, giving rise to the ashy-stem-blight phase of the disease.

Attempts to produce older plant infections in the greenhouse have been successful only under conditions of prolonged high humidity, and in the few instances where ashy-stem-blight symptoms resulted (Figs. 4 and 5), the infections seemed to have extended from local secondary lesions on leaves and petioles.

Reference already has been made to the occurrence of primary leaf infec-

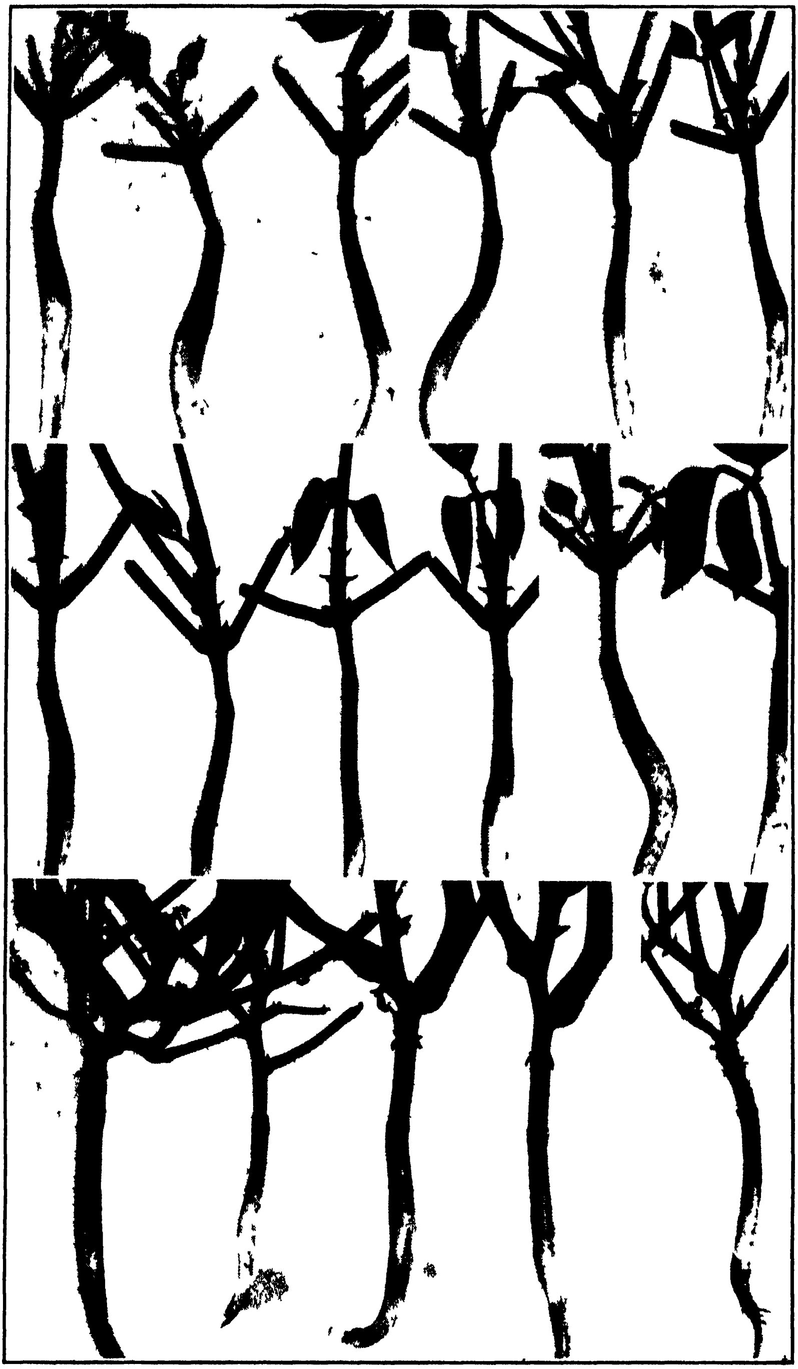

FIG. 3. Seed-borne *Macrophomina phaseoli* infection of Henderson Bush Lima in the field, showing restricted (above) and incipient (center) lesions at the cotyledonary node 20 days after planting. Below are incipient lesions that have made no further growth in an additional 4 weeks. *Ca.* ½ to ⅝ natural size.

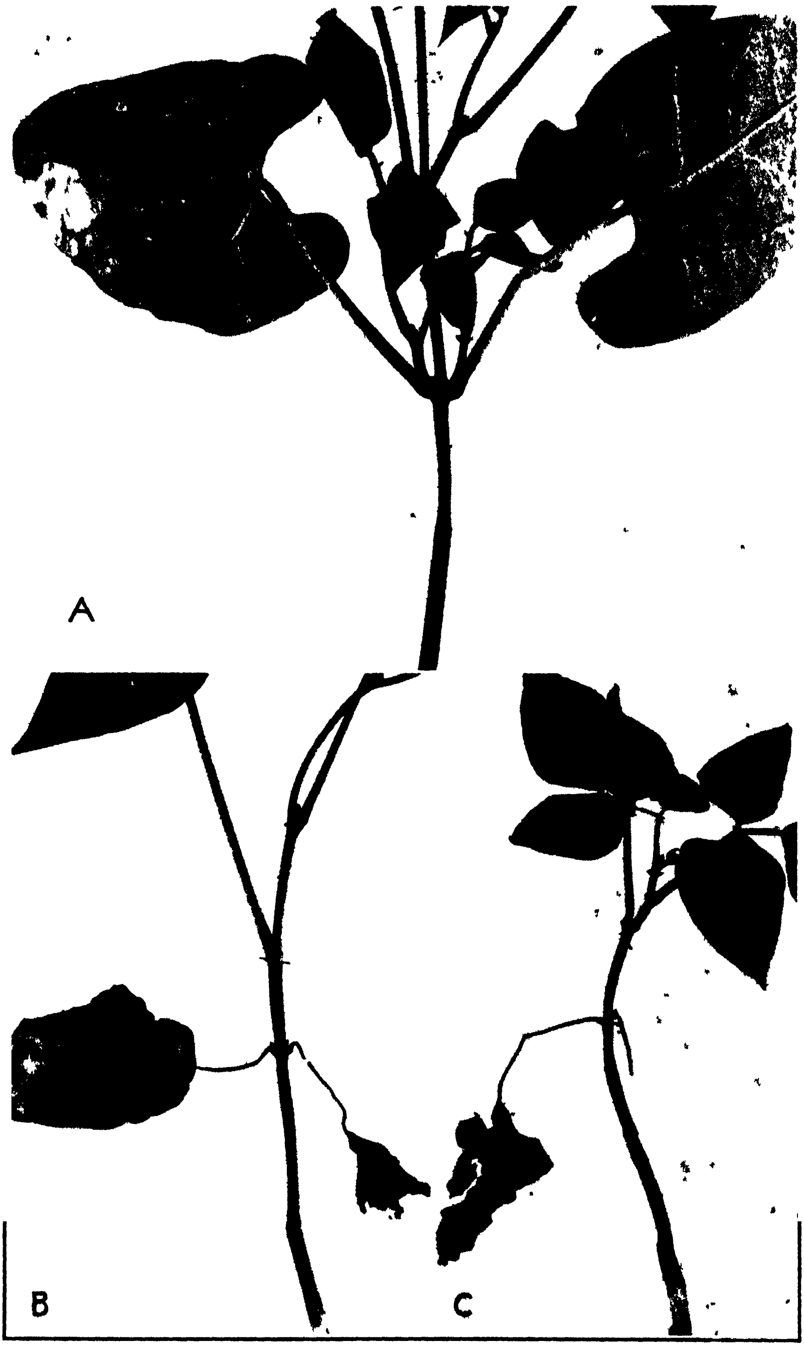

FIG. 4. *Macrophomina phaseoli* on Henderson Bush Lima beans 4 and 5 weeks old. A. Undeveloped primary infection at the cotyledonary node and secondary infection of a primary leaf. B. Primary infection at the cotyledonary node and secondary stem invasion at the primary leaf node. C. Advanced primary and secondary infection with abundant pycnidia on leaf, petiole, and stem. *Ca.* $\frac{1}{2}$ to $\frac{2}{3}$ natural size.

FIG. 5. *Macrophomina phaseoli* on Henderson Bush Lima aged 7 weeks. Primary infection at the cotyledonary node has remained dormant, while secondary infection from the primary leaf has advanced down the stem, producing ashy stem blight. *Ca.* ½ to ⅜ natural size.

tion of beans as a result of contact with infected seed coats at the time of germination. Artificial infections also have been produced on leaves of Henderson Bush Lima by inoculating with pure cultures of a pycnidial strain on agar. If held for a sufficiently long time at relatively high humidity, a leaf infection progresses through the petiole and into the stem. Pycnidia form on the decayed leaves and petioles and in the more extensive stem lesions (Figs. 4 and 5).

The most abundant pycnidium formation and most rapid development of the fungus are favored by continuous high humidity. When the infected plants are removed to a relatively dry atmosphere, the lesions make almost no growth. When restored again to the humid situation the lesions resume development.

With the combination of leaf infection and prolonged periods of high humidity and relatively high temperature, it is possible to account for the occasional severe outbreaks of ashy stem blight under field conditions in the southeastern United States. It will be necessary to make an extensive field study of the disease before the conditions favoring natural infection of older plants can be determined accurately, yet it is the impression of some who have observed the disease in the South that it develops more extensively during wet seasons.

SUMMARY

The occurrence of natural seed transmission of *Macrophomina phaseoli* is demonstrated in Henderson Bush Lima bean seed grown in Georgia. The amount of infection approximated 85 per cent in nonsterilized seed and 57 per cent in surface-sterilized seed, thus indicating that the fungus was established beneath the seed coat in over half the seed. Sterilization of seed before planting reduced subsequent seedling infection by approximately 45 per cent.

Amount of seedling injury developing from infected seed is inversely correlated with percentage of germination. Factors operating during the germination period seem to be the most important in determining the amount and extent of primary infection. Apparent correlation between temperature and infection point mainly toward its effect on germination.

Humidity is a controlling factor in secondary infection of leaves, and prolonged high humidity was necessary to produce symptoms of ashy stem blight on older plants.

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ANNUAL GROWTH[1] RATE OF CRONARTIUM RIBICOLA CANKERS ON BRANCHES OF PINUS MONTICOLA IN NORTHERN IDAHO

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INTRODUCTION

Early studies of white pine blister rust (*Cronartium ribicola* Fisch.) on western white pine, *Pinus monticola* Dougl., were concerned chiefly with determining to what extent trees of that species might be damaged by the disease. From these studies it became apparent that, except in pole-size and smaller trees growing under conditions exceptionally favorable to the rust, damage almost always would result from trunk girdling rather than from multiple twig and branch infections[3] (1; 4, p. 883; 5, p. 245; 6, p. 491). The next phase of the studies was an attempt to predict the probable extent of damage to pines and the period required for such damage to be realized. These predictions were necessarily based on infections already present in which the hyphae of *C. ribicola* would grow down the branches of the host tree and enter and girdle the trunk (1, 2, 7). Before such predictions could be made with any reasonable degree of accuracy a knowledge of the rate of promixal advance of the mycelium was necessary. Lachmund (6) quite fully summarized the data on the growth of blister-rust cankers on western white pine in British Columbia. Similar but less extensive studies dealing with the proximal growth of branch cankers have been more recently conducted in northern Idaho and the pertinent data secured are herein presented.

SELECTION OF CANKERS FOR GROWTH MEASUREMENTS

In selecting cankers for measurement the principal consideration was to obtain cankers on as wide a range of branch diameters as possible, it having

[1] The term "growth" of canker is used to define the elongation of the swollen and discolored area of bark that accompanies and bears a quite constant relationship to the progress of the fungus mycelium.

[2] The author is indebted to J. L. Mielke of the Division of Forest Pathology and to H. G. Lachmund, formerly of the same Division, for initiating the work on several of the study areas. Acknowledgements also are extended to those various members of the Division, both present and former, who assisted in the routine collection of data.

[3] Childs, Thomas W. and J. W. Kimmey. Studies on probable damage by blister rust in some representative stands of young western white pine. Jour. Agr. Research. (In press.) 1938.

been previously demonstrated that canker growth varies directly with the size of infected branch (6, p. 479; 8, p. 517). The numerical basis employed in each diameter class was roughly proportional to the natural frequency of cankers as found in the field. The second most important consideration was to choose cankers representing a wide range of developmental stages. This was to compensate for any variations in rate of growth that might have been traceable to differences in the length of time the fungus had been in the bark. All cankers selected were on trees ranging from 3 to 30 feet in height and averaging 12 to 20 feet. These canker-bearing trees were from typical stands of reproduction and were selected at random in so far as possible to do so and still secure trees bearing cankers from which a year's growth measurement could be obtained. In selecting canker-bearing branches only those were rejected that obviously would die from suppression before a year's growth could be secured on the canker. The ability of the investigator to reach a cankered branch from the ground was the only other factor influencing the position in the tree of the branches selected. Since several relatively small trees were available, however, it was possible to sample cankers in all portions of the crown, from the extreme top to the very base.

At intervals between the fall of 1932 and the summer of 1936, 18 series of cankers were marked for growth measurement in northern Idaho. These series embraced 666 cankers on a total of 167 trees at 9 different localities. On many of these cankers it was possible to secure two consecutive annual-growth measurements, but, in other cases, cankers and even entire trees and series had to be omitted for reasons enumerated in the following section. Upon the completion of the study in 1937 a total of 476 measurements of annual canker growth were available for analysis. These cankers were on 103 different trees from 15 of the original series at 6 localities. These 6 localities were all in the St. Joe region, well within the commercial white pine belt, at elevations between approximately 2500 and 3500 feet.

Table 1 presents a summary of the bases for this study and also shows the dates on which the cankers were first marked as well as those on which the initial and final growth data were taken.

MARKING AND MEASURING CANKERS

The limits of discoloration of the infected bark were used as indicating the extremities of the canker on the branch. Colley (3, p. 625) states in connection with his studies on blister rust on eastern white pine, *Pinus strobus* L., that "the irregular edge of the etiolated area marks fairly definitely the advancing tips of the invading hyphae, which generally extend a little beyond the line." Opie,[4] working with western white pine, found a positive correlation between the diameter of the canker and the extent of advance of the mycelium beyond the visible limits of discoloration.

It is evident, then, that using the limits of discoloration as the actual

[4] Opie, Robert. The relationship of *Cronarthium ribicola* to *Pinus monticola*. Unpublished Master's thesis. Univ. of Idaho. 1937.

TABLE 1.—*Bases used and important dates in the study of annual proximal growth of Cronartium ribicola cankers on branches of Pinus monticola in northern Idaho*

Localities	Basis			Cankers marked	Measurement period for annual growth	
	Trees	Cankers	Measurements annual canker growth		Beginning	End
	(No.)	*(No.)*	*(No.)*	*(Date)*	*(Date)*	*(Date)*
Deep Creek—Elk River	6	25	25	Sept. 16, 1932	July 11, 1933	July 19,1934
St. Maries River—Gold Center Creek						
Series 1	5	17	17	Sept. 13, 1932	June 6, 1933	June 14, 1934
			12		May 17, 1934	May 23, 1935
Series 2	5	22	22	Sept. 13, 1932	June 6, 1933	June 14, 1934
			13		May 17, 1934	May 23, 1935
Ruby Creek						
Series 1	16	31	31	Sept. 15, 1932	July 10, 1933	July 20, 1934
			20		May 15, 1934	May 22, 1935
Series 2	8	43	43	Oct. 5, 1933	May 15, 1934	May 22, 1935
Camp 45	25	44	44	Aug. 28, 1933	Aug. 28, 1933	Aug. 18, 1934
			32		Sept. 18, 1934	Sept. 20, 1935
Crystal Creek						
Series 1	12	38	38	July 5, 1933	Oct. 6, 1933	Oct. 10, 1934
			31		Sept. 1[illegible], 1934	Sept. 20, 1935
Series 2	14	51	51	July 6, 1933	Oct. 6, 1933	Oct. 10, 1934
			37		Sept. 19, 1934	Sept. 20, 1935
Emerald Creek	12	60	60	July 23, 1936	July 23, 1936	July 20, 1937

limits of the canker did not introduce any significant error in this canker growth study. If, for example, the hyphae extended .5 inch beyond the visible limits of discoloration on a given canker at the time of marking they should still be approximately .5 inch beyond the new limit of discoloration at the time of measurement one year later.

The limits of proximal discoloration were marked by a narrow band of bright yellow paint. The diameter of the branch at this point was measured to the nearest .01 inch. All subsequent records on the growth of the canker down the branch were secured by measuring from the original paint mark to the new extremity of discoloration. At the time of each periodic remeasurement of growth the branch diameter at the limits of discoloration was recorded. This provided the new basic diameter in the event that successive annual growth measurements could be secured on a given canker. With only one series of cankers did a measurement date fall even approximately one year from the date of marking. In all other cases it was necessary, for the sake of accuracy, to compute annual growth as between the two measurement dates that fell most nearly one year apart.

Growth measurements were not secured from every canker originally marked. The causes of loss of cankers from the basis, as listed below in order of their importance, are shown because many of the losses in this study were due to factors that need to be reckoned with in applying these results to studies of pine damage.

1. Failure to measure the canker at the proper intervals to give even approximately one year between any two measurements.
2. Destruction of trees in clearing rights-of-way.
3. Gnawing of cankers by rodents.
4. Parasitism of the cankers by secondary fungi.
5. Coalescence of cankers.
6. Killing of the host branch by the canker.

These losses in numbers were partially offset by measurements for 2 successive years on other cankers.

RESULTS

The 476 annual proximal growth measurements were summarized by branch diameter classes. Branch diameter at the base of the discolored area at the beginning of the growth measurement period was the basis for segregation. All 12 diameter classes between .01–.10 and 1.11–1.20 inches, inclusive, were represented.

Table 2 shows the basis for each branch diameter class and the average proximal canker growth over a period of a year. The standard deviation from the average growth is given for all diameter classes having a basis of 25 or more cankers.

From the basic data the regression equation for annual proximal growth over branch diameter was computed on the assumption that the data best fitted a straight-line formula. This formula was determined as being

TABLE 2.—*Average annual proximal growth of Cronartium ribicola cankers on Pinus monticola branches of various diameters in northern Idaho*

Diameter of branch		Basis				Annual proximal growth	
Range	Average	Areas	Series	Trees	Canker measurements	Average	Standard deviation
(*In.*)	(*In.*)	(*No.*)	(*No.*)	(*No.*)	(*No.*)	(*In.*)	(*In.*)
.01– .10	.10	1	1	1	2	1.38	
.11– .20	.18	4	12	35	83	1.36	±.34
.21– .30	.26	6	15	76	164	1.44	±.34
.31– .40	.35	6	15	58	93	1.52	±.38
.41– .50	.46	6	12	35	58	1.68	±.44
.51– .60	.56	6	12	19	25	1.84	±.50
.61– .70	.65	3	5	12	15	1.85	
.71– .80	.76	3	4	10	15	1.84	
.81– .90	.84	2	4	6	10	2.12	
.91–1.00	.95	2	2	5	6	2.32	
1.01–1.10	1.08	2	2	3	3	2.21	
1.11–1.20	1.13	2	2	2	2	2.41	
Totals		6	15	103	476		

Y = 1.15 + 1.116X. In figure 1 this formula is plotted to permit reading annual proximal canker growth on branches of any size between .10 and 1.20 inches in diameter.

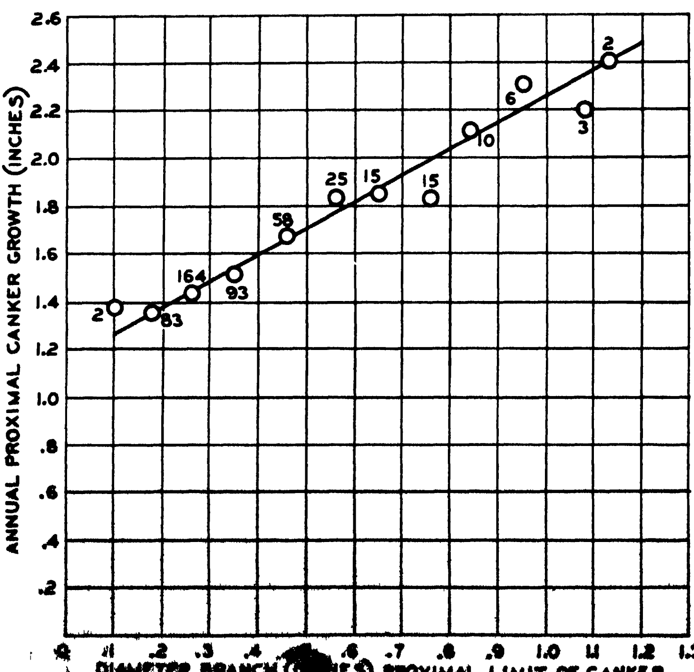

FIG. 1. Average annual proximal growth of *Cronartium ribicola* cankers on branches of *Pinus monticola* in northern Idaho. Data points are actual average values by branch diameter classes. Numeral beside each point shows the number of cankers used as a basis in determining its location.

The coefficient of correlation between canker growth and branch diameter was found to be 0.477 ± 0.035. From this figure it would seem that there are factors other than branch diameter exerting considerable influence on proximal canker growth. Branch diameter, however, is highly significant and, even when used alone, is probably satisfactory for studies whose results are desired for field application. Certainly, branch diameter is more readily measured in the field than any of the other possible influencing factors.

DISCUSSION

The number of factors that might possibly exert an influence on the growth of any individual canker are almost innumerable. The data secured have been analyzed on what has been previously found to be the most influential factor—diameter of infected branch.

The possible influence of certain of the remaining factors has been analyzed where a sufficient basis was available. In table 3 it is shown that with increasing age of the canker there is an apparent acceleration of the growth rate. A definite statement to this effect is not justifiable on the relatively small and heterogeneous basis available. It would seem justifiable, however, to conclude that the growth rate does not become less with increasing age up to at least the second season of aecial production. In this analysis cankers from all areas were used but to eliminate seasonal variation in rate of growth only cankers measured during 1933–34 were included.

TABLE 3.—*Influence of age of canker on average annual proximal growth of Cronartium ribicola cankers on branches of Pinus monticola in northern Idaho*

Diameter class	Age of canker								
	Incipients and juveniles			First and second pycnia			First and second aecia		
	Basis	Diameter	Growth	Basis	Diameter	Growth	Basis	Diameter	Growth
(*In.*)	(*No.*)	(*In.*)	(*In.*)	(*No.*)	(*In.*)	(*In.*)	(*No.*)	(*In.*)	(*In.*)
.11–.20	15	.16	1.38	30	.18	1.52	6	.19	1.61
.21–.30	23	.26	1.44	37	.26	1.63	21	.27	1.64
.31–.40	12	.35	1.52	8	.34	1.70	21	.36	1.77
.41–.50	4	.42	1.71	9	.44	1.55	18	.47	2.00

A similar analysis was made of 113 flagged[5] and 211 nonflagged cankers. No consistent superiority in proximal growth was noted for either type of canker. This is in direct accord with Lachmund's (6) findings in British Columbia. Averaging all diameters together the flagged cankers grew 1.49 inches proximally, while the nonflagged cankers were growing 1.52 inches. The average diameter of branch was .32 inch in each case. In this analysis data for a given diameter class and from a given area were not used for nonflagged cankers unless flagged cankers were available for direct compari-

[5] Cankers that have caused the death of the foliage out to the tip of their supporting branch (distally).

son and *vice versa.* It must be borne in mind, however, that none of the cankers measured were flagged at the beginning of the study. All flagged cankers suffered death of their distal ends during the course of the study.

Although the effect of age of canker and flagging have been analyzed separately, cankers of all ages and flagged cankers were included in the data on which table 2 and figure 1 are based. Obviously, these two variables did not appreciably alter the reliability of the results. The remaining factors, which might exert an influence on the rate of growth, are more or less incapable of quantitative expression. The procedure of taking a large sample over a wide range of environment, however, served largely to eliminate them from consideration in the practical application of the results. The influence of site was at least partially eliminated by marking cankers on trees from 6 distinct localities. Marking cankers on 103 different trees and an even greater number of individual branches tended to average out possible variations in rate of growth because of the vigor of the host. Possible variations in canker growth due to gross annual climatic differences were largely eliminated by taking annual measurements over 3 distinct periods; 1933–34, 1934–35, and 1936–37. The data indicated, however, that the period 1933–34 was decidedly more favorable for canker growth than was 1934–35. Direct comparison was not possible for the period 1936–37. By starting at least one series of annual-growth measurements during each of the months from May until October, possible variations in seasonal rate of growth were largely eliminated. Cankers that had grown through nodes were included as well as cankers that developed throughout the year on one internode only. Cankers suffering slight chewing by rodents were included, but severely chewed cankers were discarded.

These data, then, are presented as representing a general cross-section of the proximal growth that blister-rust cankers may be expected to make on branches of western white pine in Idaho. Although data are presented for branch diameters up to but 1.20 inches, branches of this and even smaller sizes occur abundantly in pole and even mature trees of this species. These canker-growth figures are considered to be directly applicable in studies of damage to western white pine trees up to at least 30 feet in height. For larger trees it is hard to conceive of the application of these growth figures resulting in significant errors when estimating time before damage, since, in damage studies, estimates of time that fall within ± 5 years of the actual are generally sufficiently accurate.

Lachmund's publication on canker growth (6, p. 491–499) should be carefully read before attempting to apply these growth data to studies of pine damage. In that publication the methods of application are given in detail and precautions to be taken are discussed. It must be borne in mind, however, that the average proximal canker growth in Idaho is comparable only to the growth found for the least favorable areas measured in British Columbia.

SUMMARY

During the years 1933 to 1937 annual proximal growth measurements were secured on white-pine blister-rust cankers growing on branches of western white pine within its commercial range in northern Idaho. Upon the completion of the study 476 measurements were available for summarization. This total was secured from 15 series of annual growth measurements on 103 trees from 6 different localities.

These data were segregated into branch-diameter classes for analysis, it having been previously shown that canker growth varies directly with the size of infected branch. All diameter classes between .01–.10 and 1.11–1.20 inches were represented.

The growth of cankers on branches of various sizes is shown graphically. This graph shows a straight-line trend, the growth varying from 1.26 inches on branches .10 inches in diameter to 2.49 inches on branches 1.20 inches in diameter. Growth of cankers on branches of any intermediate size can be read directly from the curve. In addition it was determined that flagging and age had no significant effect on the proximal growth of branch cankers although it appears there may possibly be some increase in rate of growth accompanying an increase in age of cankers. Other factors, incapable of quantitative expression but that perhaps influence canker growth, are discussed.

These results are considered to be of direct application in conducting pine damage studies on western white pines up to 30 feet in height. For still larger trees the application of these data should not result in significant errors.

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LEAF BLIGHT OF IRIS CAUSED BY BACTERIUM TARDICRESCENS

LUCIA McCULLOCH

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INTRODUCTION

A bacterial disease of iris leaves, which appears to be increasing in severity and distribution, has been under observation by the writer since 1924, when some diseased leaves were received from Virginia. Specimens have continued to come to the Department of Agriculture each season, but beyond determining that it was caused by bacteria, not much attention was given to the disease.

From 1934 to 1936, a number of seriously infected plants were received from Virginia, Maryland, and the District of Columbia. Most of the specimens have been sent in by growers in or near the District of Columbia, but other regions, from Alabama to Massachusetts, have been represented. All the specimens received to date (January, 1938) are varieties of the bearded group with one exception: This is *Iris cristata,* from Connecticut, sent in by the late Dr. G. P. Clinton, in 1933.

It is not likely that this is a new disease, but it is probable that the weather conditions of the past few years may have particularly favored its development in certain regions, and that observant growers are distinguishing it more frequently from other iris diseases. This disease was reported and the causal organism named and briefly described by the writer[1] in 1936. Later, Burkholder,[2] also, described it.

Except for these two reports, no record has been found in plant pathological literature of a similar iris disease. In 1931, Takimoto[3] in Japan described a bacterial leaf spot of iris and named the causal organism *Bacterium iridicola.* He sent cultures of this organism and specimens of infected iris leaves to the writer. A study of these showed that the leaf lesions and the character of the organism were unlike those of *Bacterium tardicrescens.* His description also was of an organism quite unlike *Bact. tardicrescens.*

DESCRIPTION OF THE DISEASE

Usually the first observed indications of the disease are the rather large, dark green, water-soaked spots on the leaves (Fig. 1, A). These areas are conspicuous in early morning or after any period of moist weather. A few hours of even ordinary dry weather cause a large part or even all of these areas to disappear, leaving only small, yellowish-green spots. With a renewal of moist conditions, the dark, water-soaked areas reappear.

[1] McCulloch, Lucia. An iris disease caused by *Bacterium tardicrescens*, n. sp. Paper read at the 28th Meeting of The American Phytopathological Society, Atlantic City, N. J., Dec. 28–31, 1936.

[2] Burkholder, W. H. A bacterial leaf blight of iris. Phytopath. 27: 613. 1937.

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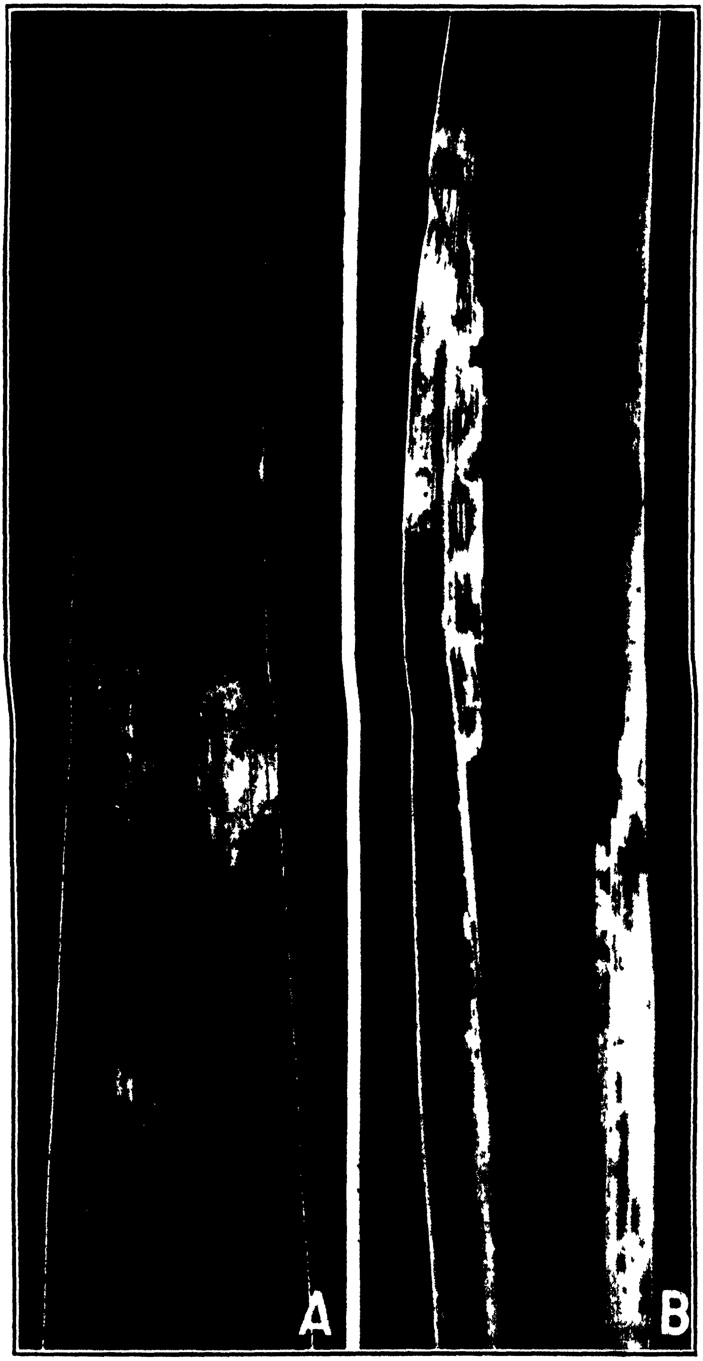

FIG. 1. Bacterial leaf blight of iris. A. Natural infection. Recent lesions with wide, water-soaked margins and isolated, water-soaked spots, photographed by transmitted light. B. Natural infection. Old lesions, dry and collapsed in the centers, photographed by reflected light. Slightly enlarged.

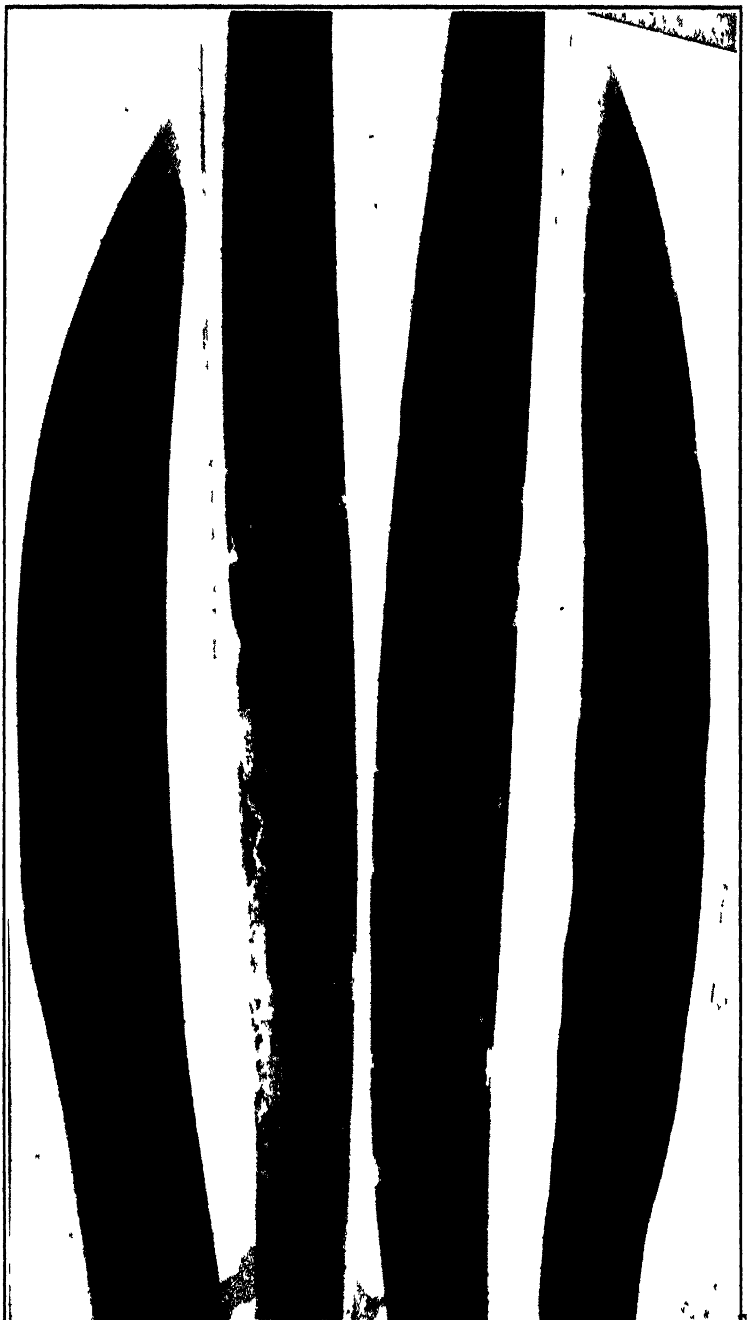

FIG. 2. Iris bacterial leaf blight. Variety magnifica. Inoculated May 27, 1936. These four leaves are from one plant. Photographed on June 8, 1936. × approximately 1.

Streaks 1 to 6 inches long of fairly uniform width and large, irregular spots are common (Fig. 2). In early stages these vary in size and shape with alterations in atmospheric humidity.

Lesions occur on all parts of the leaves above the extreme base, but they are most frequently found on the margins. Tiny, pale spots, at first visible on one side only, enlarge, become translucent, and extend through the leaf. In a dry, or even moderately dry atmosphere the spots increase in size slowly or not at all and the water-soaked margins are lacking. In such cases the infection may be unnoticed or mistaken for the common leaf spot caused by *Didymellina macrospora* Kleb. The bacteria remain alive, though inactive, as long as the leaf lives, and, in any period of sufficient atmospheric humidity, they renew growth and produce the characteristic lesions. From large active lesions there is usually a considerable bacterial exudate, a drop sometimes forming at the lower edge of the spot.

A microscopic examination of the earliest visible lesions shows that the bacteria are restricted to a very small area in the center. The water-soaked margin is at first free from bacteria, but, later, they spread into all the surrounding tissues. Progress is most rapid along vascular tissues resulting in the elongated streaks. The infected cells remain turgid for a considerable time if the atmosphere is moist and not too warm, but, eventually, the infected areas collapse and become thin and assume various shades of yellow or brown (Fig. 1, B). The rhizomes show no trace of infection.

ISOLATION

The bacteria taken directly from leaf lesions do not grow vigorously in artificial media. Four to 5, often 8 to 10 days, are required for the production of visible colonies in poured plates. Growth seldom occurs if the temperature is above 28° C., or if the agar surface becomes somewhat dry. By selecting young lesions and providing favorable temperature and humidity conditions, pure cultures of the organism are easily obtained. A peculiarity noted in isolations made directly from the leaf is that only the thickly sown plates developed colonies. It seems that single, widely separated bacteria are unable to multiply in the new and more or less unfavorable environment. If old lesions are used for isolating, the fast-growing secondary organisms, usually or often present, are very likely to develop and occupy the medium before the slow-growing parasite gets started. After several weeks in artificial media, the bacteria become better adapted to the new conditions and more rapid and abundant growth can be obtained.

INOCULATION

To test the pathogenicity of the bacteria isolated from the iris leaf lesions, iris plants were sprayed with a suspension of the bacteria in water. The first test was on outdoor plants without any protection from heat and dryness. Infection was not apparent until more than 3 weeks after inoculation and then only as very small, yellow spots. Six weeks after inoculation, during

a continued rainy period, a considerable amount of infection developed on these plants.

Repeated experiments demonstrated that a moist atmosphere maintained for 5 to 6 days or more after inoculation was the chief external factor necessary for producing good infection and development of large lesions. Most of the tests were with potted plants, which could be kept constantly moist or at least protected from drying by placing them under bell jars, in damp chambers, or covering with glassine bags. Under such conditions the infection often reaches a visible stage in 5 to 8 days, but sometimes 10 days or more elapse before there are definite signs of the disease. Once established in the tissues of the leaf and with the humid conditions continued, the bacteria multiply rapidly and may blight whole leaves in a short time.

Certain plants were inoculated and kept moist for only 2 or 3 days. Small, pale yellow lesions without the water-soaked margins were first observed 4 weeks after inoculation. Other plants not kept in a moist atmosphere following their inoculation, showed numerous small lesions in 6 to 8 weeks. Inoculated leaves often showed no visible sign of infection, but, as long as the leaves remained alive, exposure to moisture for several days would cause the development of typical lesions.

Evidently, infection, or perhaps mere entrance of bacteria to the interior of the leaf, occurs very readily; but, unless conditions are favorable, the bacteria may remain quiescent for considerable periods of time, producing visible symptoms only when suitable conditions arise.

Wounding the leaves by pricking with fine needles or by bruising was found not only unnecessary but often detrimental to infection because, under average conditions, the tissues around even tiny wounds became too dry for bacterial invasion, and, under humid conditions, soft-rot organisms often attacked the injured places.

Injection of the bacteria into iris leaves with a hypodermic needle did not produce any visible symptoms of disease in the few plants inoculated.

All visibly infected leaves may be removed from a plant, but those remaining are very likely to develop infection. When *all* leaves were removed, infection did not appear on those subsequently produced.

Iris flowers were inoculated by spraying with bacteria in water without producing any sign of disease before the flowers faded.

Inoculated rhizomes never became infected.

Infection was secured on all of the bearded irises inoculated (Lent A. Williamson, Crimson King, Miranda, Magnifica, Afterglow, Dalmatica, Mother of Pearl, Edouard Michel, and several unknown varieties).

Several other species (*Iris kaempferi, I. missouriensis, I. sibirica, I tenax* and *I. orientalis*) showed typical infections 10 to 18 days after inoculation. Two varieties of bulbous iris (*I. xiphium* ×) and ixias failed to show infection, while parallel inoculations on bearded iris produced typical lesions. The blackberry lily (*Belamcancha* sp.) became infected.

The numerous, small, isolated lesions usual in the slowly developing infec-

tions suggest stomatal invasion, and stained sections of leaves in early stages of infection show that the bacteria spread from the stomatal chamber, first, horizontally through the intercellular spaces to the opposite surface of the leaf and then, longitudinally up and down the leaf. In these sections the epidermis was intact over rather large areas of infection.

MORPHOLOGY

The bacteria are smaller than the average plant pathogens. When taken directly from leaf lesions, single rods are 0.8 to 1.8 μ long and 0.3 and 0.4 μ wide. From well-grown artificial cultures they are slightly larger, 1.0 to 2.0 μ long and 0.3 to 0.5 μ wide. The bacteria are motile by means of a single polar flagellum, 1½ to 4 times as long as the rod. Capsules are very inconspicuous or lacking. No spores or involution forms have been observed. The bacteria are Gram-negative and are not acid-fast. The writer found these bacteria unusually difficult to stain. All the usual stains and methods were tried, but the slightest washing, even with water, removed much or all of the color.

CULTURAL CHARACTERS

On beef-infusion,[4] peptone-agar plates the colonies are yellow (Mustard Yellow Ridg.),[5] circular, entire, smooth; striated interior markings, sometimes homogeneous, transparent, and viscid. Growth is slow. Colonies are usually less than 1 mm. in diameter in 4 to 6 days after inoculating plates in the usual poured-plate method. Well-separated colonies sometimes reach a diameter of 5 to 6 mm. in 3 weeks. On beef agar slants, growth is only moderate. Clouding is thin in beef broth and growth is mostly at the surface in the form of yellow pellicles and rims. Beef agar plus 0.2 per cent starch is a favorable medium for the organism. In all the beef-infusion cultures, numerous, tiny crystals form and the growth is extremely viscid, even tough and difficult to remove from the agar surface. This viscidity gradually disappears when cultures are 4 to 6 weeks old. In beef-extract agar and broth the growth is even less than in the beef-infusion media and it shows no trace of viscidity.

If the surface of the agar remains moist and the temperature favorable, growth develops as a smooth, thin, continuous layer. Under less favorable conditions, growth develops, if at all, as tiny, isolated colonies.

On potato there is a slight to moderate growth. Milk is completely peptonized in 15 to 20 days. Litmus in milk is not reduced but becomes dark blue. Methylene blue in milk is slowly reduced. In Fermi's and in Uschinsky's solutions growth is very slight. In Cohn's solution there is no growth.

The addition of 1 to 2 per cent of sodium chloride to beef broth greatly reduces growth and 3 per cent prevents growth.

In beef gelatin there is moderate growth but no liquefaction.

In blood serum, very scanty growth and no liquefaction.

[4] Beef infusion was used in all the beef media unless otherwise stated.

[5] Ridgway, R. Color standards and color nomenclature, 53 plates. (Washington.) 1912.

Starch is moderately hydrolyzed.

Nitrate reduction is positive, varying from a weak to a moderate reaction in the several isolates tested.

Ammonia and hydrogen sulphide are produced in small amounts.

All tests for indol were negative, though the bacteria grew well in peptone solution and also in tryptophane solution.

In synthetic media recommended in the Manual of Methods[6] plus various carbohydrates, growth and reactions were so slight or lacking that no conclusions regarding fermentation were warranted. In beef extract plus carbohydrates, growth was scanty to abundant. This medium plus 1 per cent glycerine produced abundant, strong yellow growth; with dextrose the growth was considerably less, only a pale yellow film on the slants with somewhat thicker and deeper yellow in the V. With sucrose and lactose still less growth developed than in the dextrose. At no time was there any indication of acid formation (Brom cresol purple was used as the indicator). Repetitions of these tests gave the same results.

The optimum pH range for growth in beef media is 6.5 to 7.5 (growth is perhaps slightly better at 7.5 than at 7.00). Growth is slight at 5.0 and at 8.0.

The minimum temperature for growth is 5° C. or lower (growth is visible in 8 days at 5° C.). The maximum temperature is 32°; the optimum 26° to 27°; the thermal death point is 44° to 46°. Growth occurred only rarely at 32°; not always at 31°, and even at 30° growth was slow, scanty and erratic. Cultures that failed to grow in 10 to 20 days at 31°, 32° or 33° would sometimes produce growth after removal to room temperature. But no growth occurred after 10 to 14 days at 34° or 35° C.

Beef-agar cultures and also sterile sand and garden soil to which broth cultures were added were stored at $-17.8°$ to $-20°$ C. Transfers made at intervals up to 17 months showed the bacteria alive and vigorous. Iris plants (bearded types) were typically infected with transfers from these long-frozen cultures.

Drops of beef broth cultures were dried on cover glasses at 27° C. Tests showed that vitality was retained for 5 to 6 days. Occasional growth occurred later but none after 10 days' desiccation.

Exposure to direct sunlight kills the organism in 5 minutes or less.

TECHNICAL DESCRIPTION

Bacterium tardicrescens

Short rods, solitary or in short chains; cells 0.8 to 1.8 μ long by 0.3 to 0.4 μ wide, or somewhat larger in well-established cultures. Motile by means of a single polar flagellum, 1½ to 4 times the length of the cell; not conspicuously capsulated. Aerobic; Gram negative; not acid-fast.

On nutrient agar, colonies are slow-growing, circular, flat, smooth, transparent, yellow; beef broth slightly clouded but with moderate yellow pellicle and rim. Gelatin is not liquefied; nitrates are reduced; starch is moderately digested; milk is peptonized; no indol

[6] Society of American Bacteriologists. Committee on bacteriological technique. Manual of methods for pure culture of bacteria (loose leaf). The Society, Geneva, N. Y., 1923 to date.

is formed; ammonia and hydrogen sulphide are formed in moderate amounts. Optimum temperature for growth, 26° to 27° C., maximum 32°, minimum 5° or lower; thermal death point, 44° to 46°. Sensitive to desiccation and to sunlight, but resists freezing for long periods.

The bacteria do not stain easily or well with the usual bacteriological stains.

Pathogenic to *Iris germanica* and various other species and varieties of bearded iris. Also to *I. sibirica, I. cristata, I. missouriensis, I. kaempferi, I. tenax, I. orientalis* and *Belamcanda* sp. (Blackberry lily). Producing leaf lesions of considerable size.

Specimens of diseased iris leaves have been deposited in the mycological collection of the Bureau of Plant Industry.

SUMMARY

A bacterial leaf blight of iris, now known to occur in a number of localities from Alabama to Massachusetts, is described. The most conspicuous symptom of the disease is the occurrence on the leaves of water-soaked areas, mostly as elongated streaks, which later collapse and become either dry or soft rotted, depending on the amount of atmospheric moisture. Rhizomes are not affected. Infection progresses slowly, except in periods of rather moist weather; but the organism survives in the leaf tissues and renews activity whenever favorable conditions arise. Pahtogenncity of the organism isolated from diseased iris was proved by artificial inoculations, which are easily affected by spraying healthy leaves with water containing the parasite. The organism has but slight resistance to desiccation and to sunlight, but it is quite resistant to low temperatures. Clean culture and exposure of the soil to sun and drying conditions and removal of all leaves in late fall or winter would probably aid considerably or prevent infections that most likely arise from bacteria that have overwintered in the soil or in old infected leaves.

Many, perhaps all, of the bearded irises are susceptible to this disease. *Iris cristata* is susceptible, *I. subirica, I. missouriensis, I. kaempferi, I. tenax, I. orientalis and Belamcanda* sp. (Blackberry lily) became infected following artificial inoculation.

Cultural characters and a technical description of the causal organism are given.

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RESISTANCE AND SUSCEPTIBILITY TO CURLY TOP IN VARIETIES OF SQUASH, CUCURBITA MAXIMA[1]

B. F. DANA[2]

(Accepted for publication April 14, 1938)

The curly-top virus has long been recognized (1, 9) as the cause of a very important disease of sugar beets in States west of the Rocky Mountains. The same virus (2, 3, 5, 7, 8, 10) has been found to attack a large number of vegetable crops throughout the same area.

[1] Data presented are based on investigations carried on during the period 1928 to 1937 by the Division of Fruit and Vegetable Crops and Diseases of the Bureau of Plant Industry, United States Department of Agriculture cooperating with the Oregon Experiment Station. Field tests for resistance have been conducted on the Umatilla Field Station, Hermiston, Oregon, where H. K. Dean, Superintendent, has assisted in general over-

Resistance to this virus is of special importance, since control measures for the insect vector, the beet leaf hopper, *Eutettix tenellus*, are lacking. Investigations of resistance in vegetable varieties to the curly-top virus have been carried on in the Pacific Northwest since 1928. Of special interest to vegetable growers and canners are the results obtained in field studies of varieties of squash belonging to *Cucurbita maxima*.

TESTS FOR RESISTANCE TO CURLY TOP

Seed Stocks

Seed of commercial varieties was obtained from seedsmen in the United States and abroad. Tests of this material as it became available were carried on throughout the period from 1928 to 1937. Much additional material was obtained through the Division of Plant Exploration and Introduction of the Bureau of Plant Industry and tested as received in 1930, 1935, and 1937.

Test Plots and Methods

Field tests for resistance have been carried on at the Umatilla Field Station. In the main, the results reported here have been obtained by exposure of test plants to natural infection. The insect vector has migrated into the plots from nearby breeding grounds in sufficient number to introduce and spread the virus generally throughout the plantings. Sugar beets, a favored breeding host for the vector, have been grown in the plots among the test plants to attract this insect and provide conditions favorable for its increase.

The natural abundance of the vector and the extreme susceptibility of the squash varieties to the curly-top virus have resulted in satisfactory elimination of susceptibles among test plants. This has made unnecessary the continued use of the inoculation methods used in early tests. Most of the varieties as indicated in table 1 were included in test plots for more than one year.

VARIETIES SHOWING LACK OF RESISTANCE TO CURLY TOP

Strains Obtained from Seedsmen

Data from tests of varieties for resistance to curly top are presented in table 1, where the percentage of plants affected is entered for all of the strains tested. Infection was uniformly very severe in all but the Marblehead variety. The percentage of crop loss in susceptible varieties closely paralleled that of disease, making it unnecessary to enter separate figures. For most of the varieties data are available for more than one year. The mate-

sight of the test plots and in providing facilities and services of laborers. Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 287 from the Division of Botany.

[2] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. R. F. Wilbur, Agent, conducted test plots in 1928 and 1929. Andrew Steiner, Agent, conducted the test plots in 1930 and assisted in 1931, 1932, 1933 and has assisted intermittently in breeding work and greenhouse work from 1934 to 1937.

rial tested in 1933 to 1937 consisted mainly of varieties not included previously or for which additional data were desired. Several of the varieties tested in 1929 did not show complete infection, but the fact that these varieties showed complete susceptibility in tests of earlier and later years indicates that in 1929 there were susceptible plants of these varieties that escaped infection.

TABLE 1.—*Tests for resistance to curly top in varieties of Cucurbita maxima*

Varieties	Percentage of plants infected for years tested									
	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
Aizu-Kust								100		
Arikara					100					
Banana	100	75	100	100		100			100	
Boston Marrow	100	100	100	100		100				
Blue Hubbard		100[a]	100				100[c]			
Beau Dessert					50				100	
Buttercup					100					
Bay State					100[a]					
Delicious		80	100						100	
Estampes					100[a]			100		
California Field								100		
Essex Hybrid		100	100		100					
Golden Hubbard	100	100[b]	100							
Gilmore			100				100			
Golden Delicious					100					
Hubbard			100	100		90			100	
Improved Hubbard	100	95							100	
Kitchenette		100	100							
Orange Marrow			100		100				100	
Mammoth Chili	100	90	100							
Mammoth Whale		50			100					
Mammoth King				100						
Mammoth Melon					100					
Sibley (or Pikes Peak)	100	90	96	100[a]						
Pink Banana							100			
Plymouth Rock								100		
Quality		90					100			
Warted Hubbard		100								
Warren			100		100					
Winnebago			100				100			
Victor			100						100	
Valpariso Red					100					
Hundred Weight					100[b]					
Vermont Hubbard					100					
Zucchetta Nana del Tronco					100					
Umatilla Marblehead	0	20[e]	4	5–7	20	0	0			
Yakima Marblehead						10	0			10[d]

[a] Two strains.
[b] Three strains.
[c] Eight strains.
[d] Crop loss, 0–5%.
[e] Crop loss, 5–10%.
(Note: Percentage of crop loss was total in most cases, but very closely paralleled the percentage of disease.)

Plant Introduction Stocks

Foreign strains of squash were included in test plots for single years only, due to limited supplies of seed. In some cases, seed germination was poor, so that very few plants were obtained. For these reasons, data on resistance of foreign strains are not reliable for every strain handled. However, the data are considered to be more truly representative of all the strains, since, with 2 exceptions, all showed complete susceptibility. Most of the affected plants died before fruit was produced. Almost no acceptable fruits were produced on any of the diseased plants. Total crop loss is entered for all but 2 of the strains, although some cull fruit was produced.

Since the one strain in 1937, which showed 20 per cent of infection and crop loss, was variable as to plant character, further tests will be necessary to evaluate satisfactorily resistance in this strain. The strain showing 75 per cent of infection and crop loss in 1931 has little promise because of the high percentage of disease (Table 2).

TABLE 2.—*Percentage incidence of curly top and consequent crop loss observed in test plots of foreign strains of squash grown in the field at Hermiston, Oregon*

Year	Strains tested	Number of strains showing stated percentage of disease		Percentage crop loss
1931	2	1	100	100
		1	75	75
1935	11	11	100	100
1937	27	26	100	100
		1	20	20

VARIETY SHOWING RESISTANCE TO CURLY TOP

The Marblehead Strains

Data for the Marblehead strains (Table 1) show very low percentage of injury compared with other varieties of this species. The figures given are for the stocks obtained from growers or seedsmen. These strains are very heterozygous for such characters as color and shape of fruit and of seed. The presence in every commercial field of an occasional plant seriously injured by curly top, while the rest of the plants are disease-free or relatively uninjured, indicates that these strains are heterozygous also for resistance to the curly-top virus.

Inbred lines, not listed in table 1, have shown varying degrees of resistance. Some have been very resistant, others have been severely injured. Those strains that have shown strong resistance have not been quite so generally high in quality as the original heterozygous strains. The isolation of additional inbred strains may be necessary to obtain a combination of the highest quality with resistance. The available inbred lines are being used in a breeding program for the production of different types of resistant squashes. They also are being used to determine the nature and behavior of resistance to the curly-top virus.

The origin of these Marblehead strains is somewhat of a mystery. The Yakima strain has been grown in the Yakima Valley of Washington for 25 to 30 years. The Umatilla strain has been grown on the Umatilla Station in Umatilla County, Oregon, for about the same length of time. The 2 strains, according to local observers, have been kept separate for this length of time. The similarity of the 2 strains, however, would suggest a common origin.

Both strains have characters distinct from all members of the Hubbard group with which the original Marblehead, introduced by Gregory, was grouped by Castetter and Erwin (4) and Erwin and Haber (6). From the standpoint of character and resistance these strains of Marblehead should be placed in a separate group.

Shape of fruit ranges from round through cylindrical-oval, elongated-oval, top-shape, egg-shape and various tapered or pointed forms. Color is variable as to ground color, striping, spotting, netting, and combinations. Of these, dark green and slate predominate as the ground color, with a small percentage of orange. Over the ground color may be spread a fine slate netting or a regular or irregular pattern of slate stripes and blotches. Slate-color fruits predominate; a very few are partially or completely orange-color.

Seed color in different fruits ranges from ivory to tan. Shape and size of seed and thickness of seed coat also are variable. Quality in these strains is good to excellent. The original Marblehead strain appears to be extinct and physical comparison with these Northwest strains for more accurate determination of relationship has not been possible.

It is indeed singular that one variety should show strong resistance, while other varieties of this species show entire lack of resistance. These strains of Marblehead present no physical characters that are not typical of the species, *Cucurbita maxima*. It is very interesting also that satisfactory quality should be combined with such strong resistance and vigor.

SYMPTOMS OF CURLY TOP ON SQUASH VARIETIES

Symptoms on Susceptible Varieties

In the seedling stage, squash plants usually succumb quickly to curly-top infection. Under high temperatures the incubation period of the virus is shortened. Exposure to drying winds, hot sun and low soil moisture greatly increases injury from the disease in plants at any stage of development.

If the young seedling is inoculated by virus-carrying leaf hoppers as soon as it emerges above the soil, it may die before true leaves appear. Death of the plant follows infection less suddenly as the plant grows older. Seedlings or young plants that die quickly do not show characteristics distinctive for this disease alone. Growth is stopped and the plant gradually withers and dies (Fig. 1, A).

Older plants may be severely injured or killed, especially if growing conditions are unfavorable. The mature leaves are not changed in form but

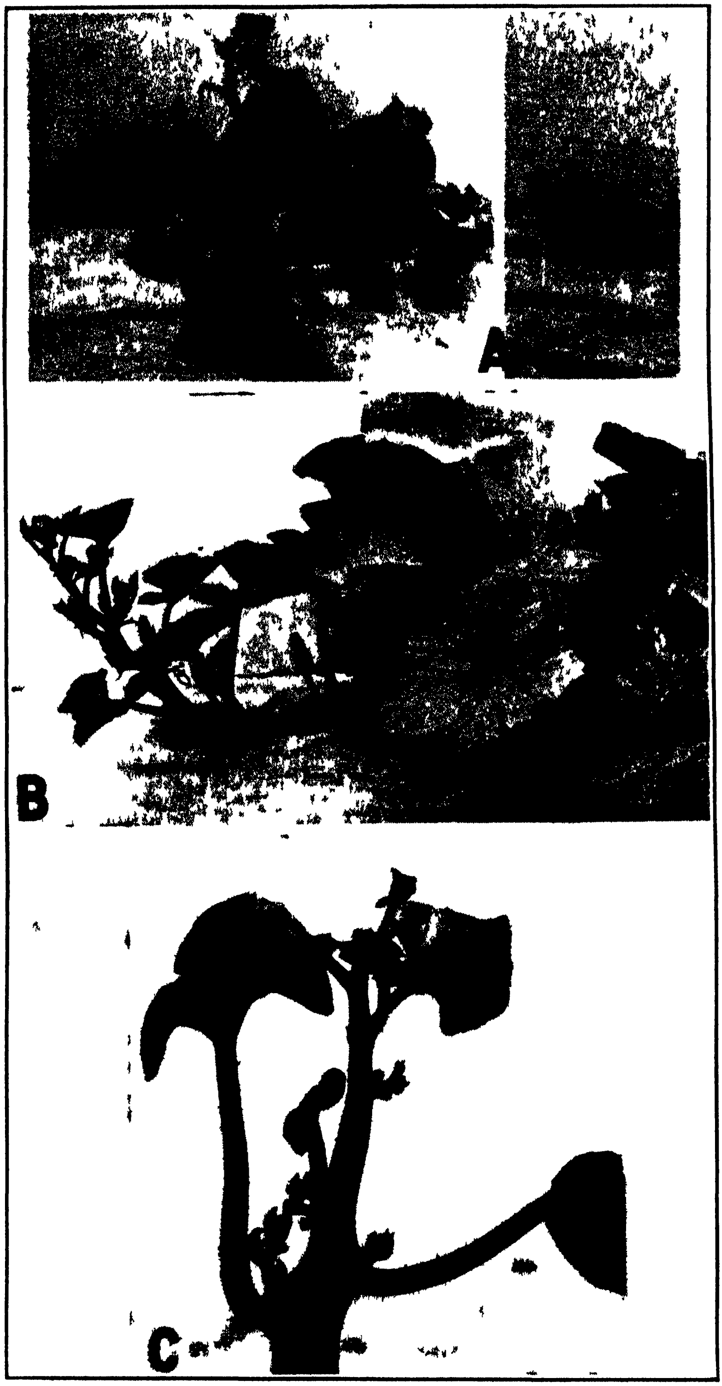

FIG. 1. A Young plants of Boston Marrow squash, *Cucurbita maxima*, dying from curly top infection. B. and C. Curly top infections on tips of runners· B, Sibley squash, *C. maxima*; C, Marblehead, *C. maxima*.

may gradually yellow and die. New growth is definitely dwarfed; internodes are shortened; and leaf blades may develop a savoyed surface or roll upward at the margin. Bending upward of the tip of the runner is very characteristic (Fig. 1, B). Blossoms on these diseased shoots usually do not set fruit. New growth on diseased plants may appear darker green than the older, deteriorating foliage.

Wilting is not characteristic of curly-top infection in squash plants. Instead, there may be gradual deterioration ending in death. Distinctive color differences by which a diseased plant may be identified are lacking. Infection late in the life of the plant reduces size of fruit and number of fruits that may mature.

Symptoms of Curly Top on Resistant Marblehead

The character of new growth at the tips of runners may be the only indication that a plant is diseased. Shortened nodes and side shoots give rise to clustered growth at the tips of runners. Dominance of the growing tip is lost and a witches' broom type of growth is formed. The individual branches often become thicker than the runner from which the abnormal structure arose.

Foliage is dwarfed and of lighter color than normal leaves. Leaf blades on the younger leaves roll upward and are somewhat more rigid than normal leaves (Fig. 1, C). Both abortive and abnormal flower development has been found on these abnormal runner-tips or "witches' brooms."

During the fall of 1937 several cases were found of partial transformation of petals and pistil of pistillate flowers to leaf-like structures. In one case the development of phyllody in the female flower was striking, with still more complete transformation of petals, style, and stigma into leafy structures. Phyllody was found only on the strongly developed "witches'-brooms" and appeared to be correlated with increased branching as a vegetative response to the curly-top virus. Similar vegetative stimulation by the virus, either in the form of witches' brooms or phyllody, has not been seen in susceptible varieties of the species, *Cucurbita maxima*.

SUMMARY

Data are presented showing that horticultural varieties of the species, *Cucurbita maxima*, exhibit extremes of both susceptibility and resistance to the curly-top virus without intermediates between these extremes.

Two strains, known as Umatilla Marblehead and Yakima Marblehead, are outstanding for resistance to the curly-top virus. Other tested varieties of this species show extreme susceptibility. The two resistant strains appear to have been developed in the Pacific Northwest. Their similarity suggests a common origin, although local observers insist that they have been grown separately for 25 to 30 years. They are extremely variable and yet nearly identical in their variations as to shape and color of fruit and seed. They

are also heterozygous for resistance, since a few plants in either strain show severe injury from curly top.

Symptoms of curly top on susceptible and on resistant varieties are described.

U. S. DEPT. OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
CORVALLIS, OREGON.

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THE PRODUCTION OF A NEW PHYSIOLOGIC RACE OF SPHACELOTHECA SORGHI[1]

SYED VAHEEDUDDIN

(Accepted for publication April 21, 1938)

By crossing monosporidial lines of *Sphacelotheca sorghi* (Lk.) Cl. the writer has obtained a physiologic race that differs from those hitherto described. Although the new race has not been found in nature, it merits attention as evidence that new parasitic races may arise as a result of crossing between haploid biotypes within a species.

The new race was produced in the following way: In 1929 Dr. L. J. Tyler obtained a collection of *Sphacelotheca sorghi* from Amarillo, Texas, and isolated individual sporidia from promycelia of several chlamydospores. The resulting monosporidial lines were inoculated into sorghum in various sexually compatible combinations, and smutted heads resulted. Dr. Tyler kindly gave the writer some of his material, and further experiments were made. The history of the crosses resulting in the production of the new race is summarized below and illustrated diagrammatically in figure 1.

[1] Paper No. 1604 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Crosses

Numerical designation	*Gametic parents*	*Color of smutted heads*
122	Tex. $B2_T$ × Tex. $C2_{\bar{s}}$	Gray
123	122 B4 × Tex. $A3_T$	Gray
124	123 A3 × 123 A4	Gray, brown, and intermediate

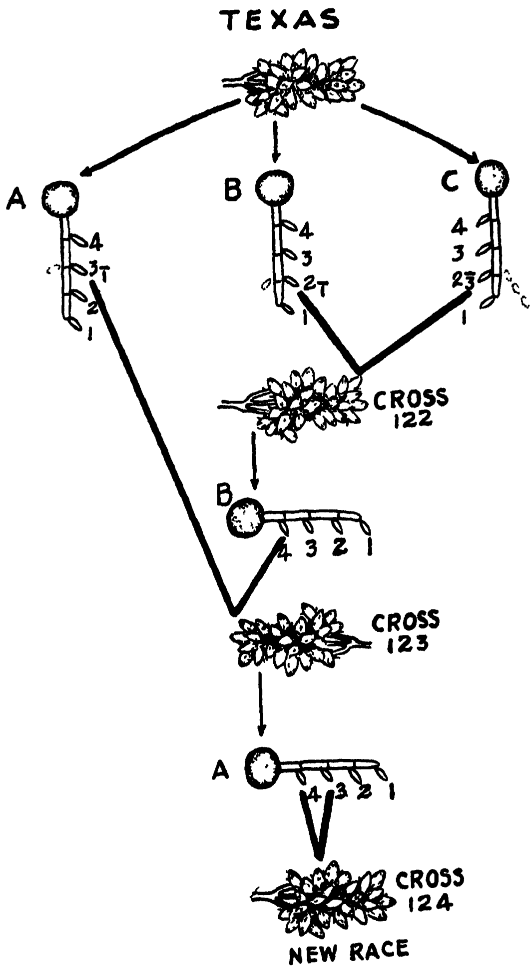

FIG. 1. Diagrammatic representation of the origin of a new race of *Sphacelotheca sorghi.*

The following explanation may make the procedure clear. The chlamydospores designated Tex. A, Tex. B, and Tex. C were obtained from a single smutted kernel of the smut collection sent Dr. Tyler from Amarillo, Texas. The monosporidial line derived from sporidium Tex. $B2_T$ was crossed with that derived from Tex. $C2_{\bar{s}}$. This was done by growing the lines separately in potato-dextrose broth, then mixing the two liquid cultures and injecting the mixture into Minnesota Amber sorghum plants by means of a hypodermic syringe. The resulting chlamydospores were designated cross 122.[2] Sub-

[2] The system of designation is shown in figure 1, but the significance of the sub-numbers may not be clear. When several sporidia are abstricted or taken from the same cell

TABLE 1.—*Percentage of smutted heads in sorghum inoculated with 5 physiologic races and "Cross 124" of Sphacelotheca sorghi*

Variety, selection, or hybrid	Accession number	Race and percentage of smutted heads[a]										
		1 (Kafir)		2 (Milo)		3 (Feterita)		4 (Feterita)		5 (Feterita)		"Cross 124" (Hegari-Kafir)
		M[a]	V[a]	M	V	M	V	M	V	M	V	V
Dwarf Yellow milo	K.B. 2515	0	0	18.0	24.0	0	1.0	0	0	0	0	0
Dwarf Yellow milo	C.I. 332	0	0	13.2	17.1	0	0	0	0	0	0	0
White Yolo	K.B. 2525	1.2	0.8	35.3	31.8	0	0	12.6	10.7	0	0	0
Pierce Kaferita	K.B. 2547	0	0	0	0	20.6	b	0	1.8	0	b	0
Feterita × Kafir	F.C.I. 8917	0	0	0	0	2.4	8.4	0	0	0	b	1.1
Feterita	S.P.I. 51989	0	0	0	0	2.6	0.5	0	0	0	0	0
Kafir × feterita	H.C. 2423	0	0	0	0	18.4	10.6	0	0	41.6	b	0
Hegari	K.B. 2518	0	0	13.6	–	0	–	–	–	–	–	6.1[c]
Reed Kafir		23.1	–	16.5	–	23.0	–	–	–	–	–	85.3[c]
Schrock		0	–	9.2	–	43.5	–	–	–	–	–	62.0[c]

[a] In column M are given the figures obtained by Melchers, Ficke, and Johnston at Kansas in 1929, while in column V are given the average percentage of smutted heads in triplicate rod rows in the writer's tests at University Farm, St. Paul, Minnesota, in 1936.

[b] Data inconclusive, but do not affect the results.

[c] Average of 2 years' results (1935 and 1936). Symbol – indicates no test.

sequent procedures were similar until cross 124 was obtained. This proved different in pathogenicity from any race hitherto described.

Forty-eight varieties of sorghum[3] were inoculated with chlamydospores from a gray head of cross 124, in the field at University Farm, St. Paul, Minnesota, in the summer of 1935.[4] As the results of these tests indicated that Cross 124 was different from the 5 races of *Sphacelotheca sorghi* previously recognized as a result of the investigations of Tisdale, Melchers, and Clemmer[5] and Melchers, Ficke, and Johnson,[6,7] further tests were made in 1936. For comparison, inoculations were made with the 5 races also, the chlamydospore material having been obtained through the kindness of Professor L. E. Melchers (Table 1).

From table 1 it is clear that Cross 124 differs from Race 5 because Kafir × feterita (H. C. 2423) is highly resistant to 124 and susceptible to Race 5. It differs from Race 4 because White Yolo (K. B. 2525) is highly resistant, while it is susceptible to Race 4. It differs from Race 3 because Pierce Kaferita (K. B. 2547), feterita (S. P. I. 51989), and Kafir × feterita (H. C. 2423) are highly resistant, whereas they are attacked by Race 3. It differs from Race 2 because the Dwarf Yellow milos and White Yolo are highly resistant, while they are susceptible to Race 2. Cross 124 differs from Race 1 because 124 attacks Hegari, to some extent. Cross 124 also differs from Races 1, 2, and 3 in its effect on other varieties, such as Schrock and Reed Kafir, on which it produced 62.0 and 85.5 per cent of smut, respectively. Cross 124 differs from Races 1 and 4 in the color of the peridium, which is gray to brownish, like that of Races 2, 3. and 5, while with Races 1 and 4 the color of the peridium is brown.

It appears, therefore, that a new race of *Sphacelotheca sorghi*, which can be designated Race 6 (Hegari-Kafir race), has been developed by crossing monosporidial lines derived from the promycelia of chlamydospores obtained from a single smutted kernel.

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of a promycelium, the first one is given the serial number of the appropriate cell, counting from the tip cell, and each subsequent one is designated by a sub-number. Thus, Tex. $C2_4$ means the fourth sporidium formed on cell 2 of the promycelium produced by chlamydospore C of the Texas smut collection.

[3] The writer is greatly indebted to Professor L. E. Melchers, Professor J. H. Parker, and Dr. J. H. Martin for furnishing seed.

[4] Vaheeduddin, Syed. Studies on the pathogenicity and genetics of some sorghum smuts. Unpublished Ph.D. thesis, University of Minnesota, 1936.

[5] Tisdale, W. H., L. E. Melchers, and H. J. Clemmer. Strains of kernel smut of sorghum, *Sphacelotheca sorghi* and *Sphacelotheca cruenta*. Jour. Agr. Res. [U. S.] **34**: 825–838. 1927.

[6] Melchers, L. E., C. H. Ficke, and C. O. Johnston. Physiologic specialization in *Sphacelotheca sorghi*. (Abs.) Phytopath. **20**: 142–143. 1930.

[7] Melchers, L. E., C. H. Ficke, and C. O. Johnston. A study of the physiologic forms of kernel smut (*Sphacelotheca sorghi*) of sorghums. Jour. Agr. Res. [U. S.] **44**: 1–11. 1932.

A Race of Ustilago avenae Capable of Infecting Black Mesdag Oats.[1]—The variety Black Mesdag has long been used as a smut-resistant parent in oat-breeding programs. Reed,[2] in 1932, and Stanton, *et al.*,[3] in 1934, have shown that certain races of covered smut, which they designate as *Ustilago levis*-Fulghum, can attack this variety. Until recently, Black Mesdag always has been resistant to loose smut when grown at University Farm, St. Paul Minnesota. In 1936, however, 2 collections of loose smut (*Ustilago avenae* (Pers.) Jens.) from Phillipsburg, Kansas, and Bartlesville, Oklahoma, produced 23 and 30 per cent of smutted heads, respectively, on this variety. In 1936 each collection was increased on Black Mesdag and on Anthony, a generally susceptible variety.

In 1937, 7 varieties of oats were inoculated with these collections and with 2 others that had been increased on Anthony and Gopher (Table 1). Chlamydospores of the Kansas and Oklahoma collections that had been produced on Black Mesdag in 1936 caused a far higher percentage of infection in this variety than those of the same collections that had been produced on Anthony. Material of the Oklahoma collection taken from Black Mesdag was much more pathogenic also to Double Cross II 20–220, (Minota × White Russian) × Black

TABLE 1.—*Pathogenicity of four collections of Ustilago avenae after having been increased on different varieties*

Source of inoculum		Variety inoculated in 1937 and percentage of infection[a]						
Original collection	Variety on which produced in 1936	Anthony	Gopher	Double Cross	Black Mesdag	Bond	Markton	South Dakota No. 165
Oklahoma	Anthony	90	43	3	15	0	1	0
	Black Mesdag	87	49	76	84	1	3	0
Kansas	Anthony	31	0	28	26	0	0	0
	Black Mesdag	43	0	26	48	0	0	0
Nebraska	Anthony	49	4	0	1	0	0	0
	Gopher	44	6	0	0	1	0	0
Indiana	Anthony	89	67	0	0	1	1	4
	Gopher	95	88	0	0	3	5	0

[a] Percentage of infection is based on the number of smutted heads in replicated eight-foot rows at University Farm, St. Paul, Minnesota, in 1937.

[1] Paper No. 1602 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Reed, G. M. Reports on research for 1932. Plant Pathology. Brooklyn Bot. Gard. Rec. 21: 42–46. 1933.

[3] Stanton, T. R., G. M. Reed, and F. A. Coffman. Inheritance of resistance to loose smut and covered smut in some oat hybrids. Jour. Agr. Res. [U. S.] 48: 1073–1083. 1934.

Mesdag, a hitherto resistant selection, than that from Anthony. This, however, was not true of the Kansas collection. Bond, Markton, and South Dakota No. 165 remained resistant to these collections regardless of the variety on which the smut had been increased.

Two other collections, from Indiana and Nebraska, were not appreciably different in pathogenicity when increased on varieties (Anthony and Gopher) susceptible to smut.

The results confirm the observations of Dillon-Weston,[4] Nicolaisen,[5] and others that the virulence of a smut collection for a resistant variety may be increased by successive inoculations of that variety, owing to the development of an increased proportion of the biotypes capable of developing on it. It is clear that a collection of smut may comprise many biotypes differing in pathogenicity for different varieties. Naturally, only those biotypes that are capable of attacking a given variety will increase on it; consequently there is a screening effect. If even a few heads become normally smutted when a resistant variety is inoculated with a collection, the variety must be susceptible to one or more biotypes in the collection, unless, of course, the variety itself is not pure; and when the variety is inoculated with its own smut the percentage of infection is likely to increase. Obviously, there are biotypes of *Ustilago avenae* that can attack Black Mesdag, which has been used extensively as a smut resistant parent in breeding work.—EDWARD K. VAUGHAN, formerly of the Minnesota Agricultural Experiment Station, now in Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, Washington, D. C.

A Seed-borne Disease of Sweetclover.[1]—In the course of the examination of the life histories of some fungi on sweetclover, *Melilotus* spp., it has been found that two species of *Ascochyta* with the same spore measurements, but with different life histories and very different pathological consequences, occur on this forage crop. The more common and well-known species is *Ascochyta lethalis* Ell. and Bart., more properly designated *Mycosphaerella lethalis* Stone from the name of its ascigerous stage. The less common species to which this note calls attention is *Ascochyta caulicola* Laubert for which no ascigerous stage has been found. While these two fungi are not readily distinguished by usual mycological procedure—the latter name has often been regarded as a synonym of the former—they are easily distinguished in culture and by the pathological conditions they effect.

The disease caused by *Ascochyta lethalis* has been called black stem by Johnson and Valleau,[2] a most appropriate name were it not for the fact that other fungi cause blackening of stems. This fungus usually fruits sparsely

[4] Dillon-Weston, W. A. R. Resistance of wheat varieties to bunt. Nature **123**: 243. 1929.

[5] Nicolaisen, W. Die Grundlagen der Immunitätszüchtung gegen *Ustilago avenae* (Pers.) Jens. Zeitschr. f. Pflanzenzüchtg. **19**: 1–152. 1934.

[1] Contribution from the Division of Forage Crops and Diseases in cooperation with the Wisconsin Agricultural Experiment Station.

[2] Johnson, E. M., and W. D. Valleau. Black-stem of alfalfa, red clover and sweet clover. Ky. Agr. Expt. Sta. Bull. 339. 1933.

and tardily on blackened areas. In strong contrast with this, the caulicolous *Ascochyta* causes bleached areas, sometimes accentuated by a brown border, and in vigorously growing plants it causes a bending of stems, which has suggested the name "gooseneck" for the disease. The fungus fruits abundantly on the lesions as soon as they appear. The common black stem fungus, likewise, occasionally produces grayish white lesions with brown borders,[3] but these can usually be distinguished by the less abundant and less conspicuous pycnidia upon them. Often both fungi occur on the same plant.

The disease caused by *Ascochyta caulicola* was described in Germany in 1903.[4] Apparently it has not been recorded in this country. Collections of what appears to be this disease are, however, in the writer's herbarium from the following sources:

Huntley, Montana. Collected Aug., 1921, by C. S. Scofield.
Urbana, Illinois. Collected June, 1931, by F. R. Jones.
Holgate, Ohio. Collected June, 1932, by F. R. Jones.
Elks County, Pennsylvania. Collected June, 1934, by George Zundell.
Lincoln, Nebraska. Collected June, 1937, by E. A. Hollowell.

The disease has been seen this summer in Kansas, Nebraska, Iowa, Illinois, and Wisconsin. In Wisconsin it has been found chiefly in pastures, where it is assumed that it must have been brought by seed, as it has not been found on roadside plants in this State except in one location. It has been found in occasional plants in seed fields in Nebraska and Illinois, but nothing is known of its frequency in such fields.

In young, vigorous, infected plants the fungus has been isolated from all parts except the root, even though lesions were found only on a portion of the stem. The fungus has been isolated also from seed from diseased plants, and in one instance plants from such seed appeared healthy when kept all winter in the greenhouse, but became covered with lesions about two weeks after they were placed out of doors in frequent rains. Thus the fungus appears to be systemic to some degree. Because of these characters, the disease deserves attention, especially in seed sources.—FRED REUEL JONES, Madison, Wisconsin.

REPORT OF THE 1938 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1938 annual meeting of the Southern Division of The American Phytopathological Society was held in connection with the meeting of the Association of Southern Agricultural Workers on February 2–4, inclusive, in Atlanta, Georgia. The morning of February 2, was devoted to a general session, when papers on a variety of plant-disease problems were given. The afternoon of February 2, all of February 3, and the forenoon of February 4 were devoted to the presentation of papers and general discussions dealing with various of the cotton-disease problems, particularly, seedling diseases, Fusarium wilt, and Phymatotrichum root rot. This part of the program was under the supervision of the Cotton Disease Council, which was considered as constituting a part of the Southern Division. Approximately 75 plant pathologists and visitors attended the general session

[3] See footnote 2.
[4] Laubert, R. *Ascochyta caulicola*, ein neuer krankheitserreger des steinklees. Arb. Biol. Reichsanstalt f. Land- u. Forstwirtschaft 3: 441–443. Illus. 1903.

on the morning of February 2, and approximately 50 attended the subsequent sessions of the Cotton Disease Council.

A root knot conference was held in the afternoon of February 4. Dr. G. Steiner of the U. S. Department of Agriculture, was the principal speaker at this conference. The remainder of the program was devoted to informal discussions of root knot problems.

An informal meeting of several members of the Tobacco Disease Council was held on the evening of February 3. The discussion was devoted primarily to policies and functions of the Council.

A short business meeting was held on the afternoon of February 2. The following officers were elected: President, G. M. Armstrong; Vice-President, V. H. Young; Councilor, A. N. Brooks; Secretary-Treasurer, Luther Shaw. A constitution for the Southern Division of the American Phytopathological Society was read and adopted.

The following resolution was adopted by the Society:

Whereas:

The Southern Division of The American Phytopathological Society has lost a friend and esteemed coworker in the passing of Dr. J. J. Taubenhaus.

Therefore be it resolved:

That the Southern Division of The American Phytopathological Society express its sympathy to the bereaved family; and, that the Secretary be instructed to forward a copy of this resolution to the bereaved family, and also to add it to the minutes of this meeting.

Signed:

V. H. YOUNG,
W. D. MOORE,
R. F. POOLE,
(Resolutions Committee)

Titles and abstracts of papers presented at the meeting follow.

LUTHER SHAW,
Secretary-Treasurer.

Eye Spot of Napier Grass. R. K. VOORHEES. Eye spot of Napier grass, *Pennisetum purpureum*, which, in the spring of 1937, was definitely determined to be caused by *Helminthosporium ocellum*, was first observed in the Agronomy fertility plots of the Department of Agronomy, University of Florida, at Gainesville. This organism had been reported previously as causing an eye spot and a ring spot of sugar cane in Cuba, and Florida. No typical ring spots were observed on the Napier grass at Gainesville, although sugar cane affected with ring spot was growing in the immediate vicinity.

The characteristic symptoms of this disease on the leaves become manifest in the appearance of reddish, somewhat oval to elongate spots, measuring 1.5–3 mm. wide by 2.5 mm. long. In cases of heavy infection the leaves wither and die prematurely, the basal leaves dying first and dropping to the ground. It is not uncommon to find the leaf sheaths and stems also heavily infected the individual spots being smaller than those on the leaves but tending to coalesce and form large, brown, sunken areas.

The disease was produced on young plants growing in the greenhouse by inoculating with a water suspension of spores from pure cultures of the fungus. Characteristic spots appeared on the leaves of the plants from susceptible stock, while no spots developed on the plants from resistant stock or the noninoculated checks.

Leaf Blights of Fig in Louisiana. E. C. TIMS and P. J. MILLS. There are two distinct leaf blights of fig in Louisiana caused by *Rhizoctonia* (or *Corticium*). Thread blight (*Corticium stevensii* (Burt)) has been prevalent for many years. Recently a Rhizoctonia blight was found that is rather different from typical thread blight. The fungus mycelium grows up the leaf petioles in a very thin layer, often causing deep cankers, then spreads fan-like on to the leaves. The leaf tissues turn brown and often fall out, producing a shot-hole effect. The leaves quickly shrivel and fall off if conditions are favorable, seldom adhering to the twigs as in the typical thread blight. The Rhizoctonia blight produces no powdery gray to brown mycelial mats on the lower surface of the leaf—basidial stage—and no typical threads or sclerotia, as in thread blight. The two blights have not been observed on the same tree. A rapid-growing *Rhizoctonia*, similar to the forms found widely distributed in the soil, was readily isolated from diseased fig leaves and its pathogenicity demonstrated. This fungus differs in appearance, growth rate, etc., in culture from the rather slow-growing form associated with thread blight. Neither of the organisms has produced the basidial (*Corticium*) stage in culture, under our conditions.

Problems in the Germination of Cottonseed. D. M. SIMPSON and G. M. STONE. Seedling stands of cotton are affected by the sensitivity of the planting-seed to adverse condi-

tions. Sensitive seed lots quite often germinate well in the laboratory when tested by the standard method of cottonseed germination, but fail to produce good stands when planted in the field. A new laboratory method of detecting the degree of sensitivity has been tested at Knoxville, Tennessee, and the results show close correlation with field behavior. Essentially, the method is as follows: The seeds are delinted with sulphuric acid, placed in water and vacuumized for five minutes at a negative pressure of 27 inches of mercury, then removed, drained of surplus water, and placed on moist paper towelling for germination at the usual 20–30° C. alternate temperature as in the standard method for cottonseed germination. The weak seeds fail to germinate after being subjected to the vacuum treatment. Studies indicate that sensitivity may be closely associated with the texture or structure of the seed coat.

Lightning Injury to Cotton. A. L. SMITH. Lightning-struck spots in cotton fields vary considerably in appearance. Perhaps the commonest type of injury is the killing of all vegetation in roughly circular areas with marginal intermingling of living, dead and injured plants. Quite commonly, however, little or no sudden killing occurs and exact centers of struck spots cannot be determined. This latter type of lightning injury is particularly confusing to growers, since noticeable symptoms do not appear until sometime later when scattered plants commence wilting and dying. Areas 300 ft. in diameter thus affected have been observed. Presumably, the magnitude of the discharge, soil moisture, and soil type largely determine the extent of injury at the center and the distance of lateral spread.

Plant symptoms are similar in all cases. Plants not killed outright show injury at or near the soil line, which consists essentially of a girdling of the stem. Presumably, the cambium and phloem tissues are killed in a narrow band. Subsequent developments are root starvation, a collar-like enlargement above the injury with adventitious root development, reddening of the foliage and subsequent wilting and dying of the plant.

Comparative Injury of Root knot Nematodes to Different Varieties and Species of Cotton in Control Experiments Under Irrigation. C. J. KING. Upland varieties of cotton show little injury in soils having a moisture equivalent above 18. At 16 or less the stands often are reduced, and mature plants may appear unhealthy. Egyptian, American Egyptian, Peruvian, and Sea Island varieties are more susceptible. The F hybrids of upland and American Egyptian varieties are intermediate in susceptibility. The ratio of yields for Acala and Pima on adjoining strips of root-knot-free land has averaged 1.8 over a period of several years. Similar tests on infested soils gave a ratio of 3.25. Frequent irrigations during seedling development reduce injuries to Pima cotton, especially when ammonium sulphate is added to the water 1 part in 10,000. Heavy applications of ammonium compounds, calcium cyanamid, and organic fertilizers have been beneficial in reducing nematode injuries. The effects on highly calcareous soils are not lasting. Under Arizona conditions clean fallowing for 3 to 4 years, combined with frequent deep tillage in summer, nearly eradicates nematodes. Reinfection of the treated areas is, however, rapid unless precautions are taken. Rotation of cotton and alfalfa, with each crop maintained for 2 or 3 years is a practical method of control in some areas.

Further Studies of Crinkle Leaf, a Disorder of Cotton Plants Prevalent in Lintonia and Olivier Silt loam Soils in Louisiana. D. C. NEAL and H. C. LOVETT. The disease of cotton known as crinkle leaf, prevalent in certain Lintonia and Olivier silt loam soils in Louisiana, has been found associated with high acidity, calcium deficiency, and manganese toxicity. This disease results in the formation of puckered, mottled, partially chlorotic, and variously distorted leaves in the early stages; fasciation of branches; and the development of distorted floral buds, flowers, and bolls. Soil from areas in Louisiana affected with crinkle leaf has a consistently lower pH and contains significantly larger amounts of manganese than soil from healthy areas. The disease was produced in cotton plants in sand cultures by the addition of increasing amounts of manganese sulphate. It is readily corrected by calcium and other basic carbonates.

Results of Seed-treatment Tests with Cotton in 1937. S. G. LEHMAN. An attempt was made to determine the relative effectiveness of Ceresan and New Improved Ceresan as seed-treatment materials for cotton. These commercial preparations were applied in such quantities as to add to the seed equivalent units of the active ingredient in the preparations. Two plantings—medium early and medium late—were made.

New Improved Ceresan increased seedling emergence significantly more than Ceresan in the medium-early plantings on Cecil sandy-loam soil at Raleigh and on Norfolk sandy loam near Rocky Mount when the amount of material applied was sufficient to give adequate coverage. In the medium late planting these 2 preparations gave approximately

equal increase of emergence, the increases being barely significant at Raleigh and decidedly significant at Rocky Mount.

Both materials increased the yields by an approximately equal percentage. These increases were generally significantly large when sufficient material had been added to give good coverage of seed except in the medium late planting at Rocky Mount where the increases barely approached significance.

Zinc oxide, copper oxychloride "A," and cuprocide gave significantly large increases in emergence at Raleigh and Rocky Mount in the early planting. In the medium late planting these materials gave small and generally insignificant increases at Raleigh, but larger and significant increases at Rocky Mount.

Ceresan greatly reduced the percentage of sore shin and cotyledonary lesions of angular leaf spot on seedlings. This effect on angular leaf spot was still evident on plants in the late summer.

Results from Treating Cottonseed with 2 Per Cent Ceresan. U. R. GORE. Cottonseed-treatment tests were conducted by the Georgia Experiment Station in 1937 at 3 locations in south Georgia and one in the Piedmont. In these tests a small factorial design with 2 varieties, nontreated and treated with 2 per cent Ceresan was used. Dixie 14–5 and Coker Clevewilt 6 both germinating 95 per cent were the varieties used in south Georgia. Dixie 14–5 germinating 95 per cent and seed of another variety germinating 30 to 38 per cent were used in the test in the Piedmont. Tests were drill planted at Readsville on March 31, at Hawkinsville and Cordele, April 2. The weather was cool and generally unfavorable for cotton during April. The test at Experiment was a medium late drill planting, May 6. Weather conditions were ideal for cotton.

Results reported here are from data on one year's tests only. Data were secured on (1) seedling stands before chopping, (2) plants per acre at time of harvest, and (3) yield of seed cotton per acre. Analysis of variance of the data shows no significant increases in stand before thinning for Ceresan treated seed at any location. A significant increase in number of plants per acre at harvest was obtained from Ceresan at one location and a significant decrease was obtained at one location. Increases in yield of seed cotton from Ceresan were obtained in 5 out of 8 comparisons. The differences in yield were not statistically significant for Ceresan treatment at any location.

Cottonseed Treatment for Stand Improvement. D. C. NEAL. In cottonseed-treatment experiments conducted in 1937 at Baton Rouge and St. Joseph, La., for the improvement of stands, it was found that Cerasan at the rate of 3 ounces per bushel and Improved Ceresan at a rate of 1 ounce per bushel were about equally effective. Both of these dusts at the above rates were superior to copper oxide.

Delinting cottonseed, either mechanically or by means of sulphuric acid, gave no improvement in stand during the season of 1937 at Baton Rouge, but mechanical delinting did result in a higher yield of seed cotton. Sulphuric acid delinting, on the other hand, gave a significant reduction in both stand and yield in these tests and does not appear promising for Louisiana conditions.

In districts where growers prefer the hill-drop system of planting, mechanical delinting and subsequent dusting with Ceresan or Improved Ceresan at rates of 3 and 1 ounce per bushel, respectively, will likely result in more uniform stands and better yields.

Cottonseed Treatment Gives Larger Yield. W. C. NETTLES. A $1,500,000 increase in the State's cotton crop by seed treatment for only 250,000 acres points the way to still greater results in 1938 and the future.

One acre out of every 6, or 250,000 acres, planted to cotton in South Carolina in 1937 was planted in seed treated with mercury dust known under the trade name of Ceresan. Results indicate more plants and healthier ones, and increased yield. Twenty four per cent more plants were present on treated rows than on nontreated rows at chopping time.

The most striking result obtained was the reduction in sore shin. Over 60 per cent of the plants grown from nontreated seed had sore shin, while only 16 per cent of those grown from treated seed were so affected, and then very slightly.

On 115 farms scattered throughout South Carolina, treated seed produced 16.7 per cent more bolls than nontreated, which means an extra 205 pounds of seed cotton per acre.

While these figures are based on partial returns, it appears certain that complete returns will strengthen the results, and that the seed treatment on the 250,000 acres alone will increase the value of the 1937 cotton crop by $1,537,500.

The Reaction of Cotton Leaves to Hypodermic Injections of Fusarium vasinfectum. A. L. SMITH. Preliminary studies of methods for differentiating cotton plants for reaction to *Fusarium vasinfectum* indicated possibilities of producing leaf symptoms by hypo-

dermic injections in stems near the terminal bud or in leaf petioles. Symptoms produced were similar to those on naturally infected leaves except that small portions of leaf blades generally were involved. Limited areas becoming browned were bounded by discolored veins that, when cultured, yielded the organism previously introduced. Further inoculations were made in the greenhouse, using 6 varieties varying widely in known reaction. The percentage of inoculated leaves showing wilt symptoms for Clevewilt (resistant) and Half and Half (susceptible) was 88.9 and 83.3 per cent, respectively. Other varieties showed lower percentages of infection. Since these two varieties represent the extremes in known reaction of the varieties studied, it was concluded that no relation exists between natural field infection and the appearance of leaf symptoms following hypodermic injection in leaf petioles. A more extensive study under field conditions, using the same varieties, confirmed the above results.

Technique of Artificial Inoculation with Fusarium vasinfectum. D. C. NEAL. In additional studies of wilt infection technique with cotton seedlings in the greenhouse, results have been secured with 4 varieties of cotton, namely, Delfos 2323, Clevewilt, Dixie Triumph 12, and Half and Half, which appear significant for resistance and conform in some degree to the field behavior of the varieties. Similar trends of resistance were secured when the plants were grown in 2 soil types after being inoculated with 5 different isolates of the fungus grown simultaneously in Czapek's solution. Cultures of the fungus grown on a 4:1 mixture of oats and wheat and subsequently mixed with sand to give a mixture of 5 per cent of the fungus inoculum have been found to be satisfactory for field inoculations. In studies of the effect of pH on wilt infection, it was found that higher infection was obtained at pH 5.5 to 5.7 than in the alkaline ranges or in those that tended toward alkalinity in Ruston sandy loam. In Lintonia silt loam the most favorable soil moisture for infection was 60 per cent of the water-holding capacity.

Air-dried Oat-wheat Mixture for Contaminating Soil with the Cotton-wilt Organism, Fusarium vasinfectum. A. L. SMITH. Comparative studies of freshly prepared and air-dried oat-wheat mixture for contaminating field soils with *Fusarium vasinfectum* indicates an equal or slightly superior infective power of the air-dried mixture. The air-dried material was produced and dried during the winter months and stored until spring. Both materials were produced in 24-gauge, galvanized iron containers of 1 bu. capacity equipped with 4″ screw top with ½″ opening for plugging, a ¼″ opening for pressure equalization during sterilization, and handles.

The mixture, consisting of 3 parts oats and 1 part wheat, was placed in the containers hydrated by soaking 12 hours in an excess of water, drained, sterilized and seeded with 250 cc. of spore suspension. After 10 days' incubation the mixture was emptied in bushel baskets and incubated an additional 48 hours before placing in seed-drying trays. After drying to 14 per cent moisture, the material was resacked and stored until used. Early field applications placed in deep furrows (4″–8″ below the seed level) and well mixed with the soil gave best results. When applied at the rate of 685 pounds per acre, a maximum of 75 per cent of dead or wilted plants was obtained with Half and Half variety on soil previously having a very light wilt infestation.

A Promising Wilt-resistant Long, Staple Cotton. D. C. NEAL and C. B. HADDON. A selection of Delfos cotton (Delfos 2323–965–425) made at the Northeast Louisiana Experiment Station in 1934 has exhibited marked resistance to Fusarium wilt in tests conducted for the past 3 years on heavily infested soil at Baton Rouge, Louisiana.

At Baton Rouge this selection produced over 1100 pounds of seed cotton per acre, and in a comparative test with 6 standard varieties at St. Joseph, La., in 1937, it ranked second in production, yielding 2246 pounds of seed cotton per acre. On bluff soil the staple averages 1⅛ inches and from 1 5/32 to 1 3/16 inches in the Delta, with about 32.5 per cent of lint.

Fertilizers in Relation to Incidence of Wilt as Affecting a Resistant and a Susceptible Variety. JAMES B. DICK and H. B. TISDALE. In 1937 the Division of Cotton and Other Fiber Crops, cooperating with the Alabama Experiment Station, began a study to determine the effect of different combinations of nitrogen, phosphate, and potash upon the incidence of cotton wilt. On the basis of 600 lb. per acre, 3 levels of nitrogen (0, 6, 12), phosphorus (0, 8, 16), and potash (0, 4, 8) were used in a factorial design, thus giving 27 fertilizer combinations. Two varieties, Cook 307 (resistant) and Half and Half (susceptible) were used. The experiment was planned to continue for 3 years. The first season's results indicated that:

1. While there is a high inverse correlation between yield and the number of plants affected by wilt, some fertilizer combinations have an additional effect on yield.

2. Applications of nitrogen and potash alone, and especially in certain combinations, effectively reduce wilt and increase yields. There is a differential response of varieties to fertilizers under conditions of severe wilt. The use of phosphate materially increases

the incidence of wilt, particularly so in the absence of adequate amounts of nitrogen and potash. Even when the latter are present it appears that increased wilt largely nullifies the beneficial effects of phosphate on yield.

Cotton Varieties in Relation to Cotton Wilt. H. B. TISDALE and JAMES B. DICK. The Alabama Experiment Station, cooperating with the Division of Cotton and Other Fiber Crops and Diseases, began studies in 1936 on the relation of cotton varieties to the cotton-wilt diseases. The results of these studies for 1926 and 1937 indicate:

1. No evidence for the existence of different physiological strains of the wilt organism in Alabama despite marked differences in virulence for location and conditions. Classification of varieties into resistant, highly tolerant, weakly tolerant, and susceptible groups, based on relative resistance appears to be unaffected by severity, although in one test the resistant group may have as much wilt as the weakly tolerant group at another location.

2. The varieties of cotton tested differ in their response to varying applications of potash. Resistant varieties are benefited more by moderate applications of potash than are the susceptible varieties. The latter apparently require more potash and are indifferent to excess applications, while resistant varieties require less potash and show injury and delayed maturity with excess applications. A gradation of these requirements from the most susceptible to the most wilt-resistant varieties suggests relationship between wilt resistance and ability to use potash.

Field Studies on Fusarium Wilt of Cotton in Arkansas. The relation of "wilt" and "total infection" as influenced by potash fertilization. E. M. CRALLEY and W. H. THARP. Three varieties of cotton—resistant, tolerant and susceptible—were tested in a randomized arrangement at Ozark in 1936 and LaGrange in 1937. At Ozark, potash was applied in the seed bed 10 days prior to planting, and as side dressing 1 month and 2 months after planting. But 1 application of potash was made at LaGrange, it being placed in the seed bed 21 days prior to planting. Wilt and infection data were obtained by pulling and examining plants at different periods during development.

The data were recorded in terms of both wilt and infection, and a wilt-index method has been employed to facilitate measurement of relative disease severity.

The results from the 2 years' work indicate that under the conditions of these experiments: (1) infection occurs progressively with advance in season in resistant, tolerant, and susceptible varieties; (2) significant differences in relative disease severity between potash-fertilized and nonfertilized plants are accentuated only late in the growing season; (3) applications of potash after planting were decreasingly effective with lapse of time between planting and application.

The Effect of Heavy Metals and Minor Elements upon the Growth of Phymatotrichum omnivorum in a Nutrient Solution. L. M. BLANK. A study has been made of the effect of some heavy metals and minor elements upon the growth of *Phymatotrichum omnivorum.* Included were copper, iron, manganese, zinc, aluminum, boron, silicon, cobalt, fluorine, iodine, lithium molybdenum, nickel and mercury. To a nutrient solution purified by treatment with calcium carbonate, the metals or minor elements were added at various rates. The dry weight of the fungus mat after 28 days incubation at 28° C. has been used as the criterion of the effect of the various elements.

The factorial design was employed. The data are being treated statistically. From the analysis so far made, high significance is evident for the stimulating or inhibiting effects of certain metals at given concentrations. The design permits evaluation of their effects independently and in various combinations. Slides showing the analyses of two experiments illustrate significant main effects and interactions resulting from combinations of 2 or more metals. Since the design may be of interest to other investigators, its efficiency and the method of analysis is shown. Until more of the data are analyzed, conclusions will not be attempted for the possible rôle of these elements nor for their necessity or concentration for optimum growth requirements.

Some Studies on Phymatotrichum Root Rot. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE. Monocotyledonous plants apparently owe their general immunity from root rot to an acidic, ether-soluble material in the roots. Fractions containing this have been prepared from onion, gladiolus, cane, canna, Hemerocallis, and Johnson grass. A possible perfect stage appeared in transfers of *P. omnivorum*, previously carried for 6½ years in culture. In drop cultures, hyphae of this lamellate or poroid basiodomycete anastomosed with those of known *P. omnivorum.* Laboratory experiments showed that pentachlorethane, tetrachlorethane, and xylene were highly toxic and readily penetrated moist soil. However, these volatile fungicides have not eradicated root rot from infested plots and are not, as yet, recommended for practical use against Phymatotrichum root rot.

Soils originally acid or acidified by various chemicals are less favorable for infection or survival of root rot than neutral or alkaline soils. In acidifying soils to attempt control of root rot, the reaction of the surface soil layer appeared particularly significant. Barriers of sorghum plants prevented spread of root rot in cotton fields, and are recommended to block invasion of uninfested areas and to border experimental plots.

Cotton Root Rot in Texas in 1937, and Conditions Affecting Its Local Prevalence. WALTER N. EZEKIEL. Estimates of losses to the 1937 cotton crop from Phymatotrichum root rot were made on September 20–24 for 770 fields, in 22 counties. Mean percentages of plants killed in different counties varied from 0 to 29.1 per cent. For different portions of the State, reduction in yield was estimated at 0 to 12.1 per cent, and for the State, about 5.9 per cent or over 300,000 bales. In 10 selected counties with soils approximately equally favorable for the disease, root-rot losses correlated highly with April, May, June, and July rainfall and normal mean annual temperature. Increase of 1 inch of rainfall in April or May was associated with increase of about 14 per cent of root rot; in June with about 20 per cent; and in July with about 32 per cent. Differences in August rainfall were apparently without effect. Temperature increase of about 1° F. was associated with an increase of about 1.1 per cent of root rot. The disease is not prevalent in sections of Texas with mean normal temperatures below about 60° F.

REPORT OF THE TWENTY-SECOND ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twenty-second annual meeting of the Pacific Division of the American Phytopathological Society was held in Balboa Park, San Diego, California, June 21–23, 1938, in conjunction with the meetings of the Pacific Division of the American Association for the Advancement of Science.

The three half-day sessions for the presentation of papers were attended by from 30 to 50 pathologists from California, Oregon, Washington, Arizona, and Hawaii. Twenty-six papers were presented, the titles and abstracts of which follow this report.

Wednesday afternoon was devoted to excursions in the vicinity of San Diego, the largest group visiting the Scripps Institution of Oceanography at La Jolla. A short informal symposium on the teaching of plant pathology was held Thursday afternoon. The same topic has been suggested for a more extensive program at the next annual meeting.

At a business meeting the following officers were elected:

W. T. HORNE, President, University of California Citrus Experiment Station, Riverside, California.

B. F. DANA, Vice President, U. S. Department of Agriculture, Corvallis, Oregon.

L. D. LEACH, Secretary-Treasurer, University of California, Davis, Calif.

EUBANKS CARSNER, Councilor, U. S. Department of Agriculture, Riverside, California.

M. W. GARDNER and L. D. LEACH, Members of Council of Pacific Division of A.A.A.S.

The next annual meeting will be held at Stanford University, Palo Alto, California, during the third week of June, 1939.

Movement of the Virus of Sugar Beet Mosaic. C. W. BENNETT. The virus of beet mosaic moved inward from the distal end of beet leaves a distance of 12 inches in 72 hours. No virus was obtained from opposite noninoculated leaves of these plants over a period of 4 weeks following inoculation. Invasion of large crowns from inoculated small shoots of the same plants was relatively slow. Movement in the reverse direction was much more rapid. When infection was produced in one shoot of a plant having 3 crowns separated by distances of 12 inches or more, the virus required a relatively long time to invade either of the other 3 shoots when they were growing normally. However, when 1 of the noninoculated shoots was defoliated or placed in the dark for 5 days the virus invaded it and produced symptoms within a short time. Using plants with 3 shoots, one was inoculated with the virus of curly top and another with the virus of mosaic, the third shoot being held as a check on relative rate of movement of each of the 2 viruses into it. Mosaic symptoms appeared on the inoculated and check shoots in average times of 8 and 51 days, respectively. Symptoms of curly top appeared in average times of 15 and 181 days, respectively. Other evidence indicates that when the 2 viruses are introduced simultaneously into a shoot of a plant bearing 2 or 3 shoots, the virus of mosaic invades the noninoculated shoots ahead of the curly-top virus. This is believed to be due to the ability of the virus of mosaic to move in both phloem and parenchyma, whereas movement of the virus of curly top probably is confined to the phloem.

Serological Differentiation of Citrus Red Scale, Aonidiella aurantii and Citrus Yellow Scale, A. citrina. F. R. BUSHNELL and L. J. KLOTZ. By precipitin technique it was found possible to distinguish between *Aonidiella aurantii* and *A. citrina.* Eight grams of the living *A. aurantii* were ground in a mortar with quartz sand and extracted by shaking in a 100 cc. solution of 10 per cent NaCl. The extract was dialyzed to remove the salt, and the resulting precipitate was dissolved in 0.85 per cent NaCl solution. This material was injected intravenously and intraperitoneally into rabbits at 3-day intervals and the antiserum recovered 8 days after the last (8th) injection. Antigen of the red scale (*A. aurantii*) in a dilution as high as 1: 13 gave an abundant precipitate with this antiserum. Antigen of living yellow scale (*A. citrina*) gave no precipitate at any dilution used. One lot, labelled yellow scale, did give a reaction in a dilution as high as 1: 6, but later proved to be a mixture of red and yellow scale. While recent studies show that the 2 species may now be readily distinguished by microscopic morphology, thus superseding the possible usefulness of serological technique in taxonomy of these insects, the discovery that such closely related insects are seriologically different suggests a usefulness of the technique in this field. Extracts of dead Argentine red scale (*C. aurantii*) from tung oil trees and dead Asiatic red scale (*C. aurantii*) from roses gave no precipitin reaction with citrus red scale antiserum at any dilution. This suggests that if these 3 lots of scale are the same species, food or death (or both) of the insects alters their proteins.

The Present Status of Curly-Top Resistance in Sugar Beets. EUBANKS CARSNER. The curly top-resistant sugar beet, variety U. S. 12, combines very high resistance with satisfactory sugar content. In a test under severe curly-top exposure its calculated acre yield of indicated-available *sugar* was 1.7 tons as compared with a total acre yield of *roots* of 1.6 tons from the European variety, R. & G. Old Type. Under less severe exposure, where R. & G. Old Type yielded 10 tons of beets per acre, U. S. 12 gave 23.7 tons of beets and 3.2 tons indicated-available sugar per acre. U. S. 12 bolts readily with fall planting in California, but, where spring planting is practiced, it shows little or no bolting.

U. S. 33 is characterized by moderate curly-top resistance and high sugar percentage. The bolting tendency is high.

U. S. 14 is low in bolting tendency and moderately resistant to curly top. Adaptability of the variety is restricted because it is very susceptible to downy mildew.

The 3 varieties mentioned will continue in commercial use for at least the next 2 years and the extent of their use will be in the order they are listed here.

Further Studies of the Host Relationship of Peach Mosaic in Southern California. L. C. COCHRAN and LEE M. HUTCHINS.

A Study of the Pathological Anatomy of Psyllid Yellows with Special Reference to Similar Changes in Sugar Beets Affected with Curly Top. J. R. EYER and MAYBELLE MILLER. In a histological study of material from plants affected with psyllid yellows, 4 abnormalities were found. Phloem necrosis occurred in stems, stolons, roots, and lateral rootlets, being most severe in stems and stolons. Pseudocalluses in the sieve tubes, and an increase in number of sieve plugs also were observed. Nuclear changes were prominent in the companion cells, phloem parenchyma, pericycle, and cortex. These manifested themselves in the form of the flaky appearance of the nucleoplasm, beaded membrane and hypertrophy and contortion of the entire body. None of these abnormalities was observed in histological sections from healthy plants.

Artschwager has reported very nearly the same malformations in his study of sugar beet seedlings affected with curly top.

Transmission of Psorosis of Citrus. H. S. FAWCETT. Since the discovery of leaf symptoms of psorosis in 1933, on the same trees with the previously known scaly bark symptoms, much new evidence has accumulated as to the apparently limited means of transmission of psorosis. Rubbing with crushed tissue or extracts of diseased plants, injected filtered extracts, etc. have failed to transmit this disease. No vector has yet been found. Investigations indicate that transmission from contiguous trees in nursery rows or in the orchard is unimportant in California. Transmission experimentally has been made only by the fusion of live cells of the diseased buds, bark, or root grafts with the live cells of healthy plants. Recognition of the leaf symptoms has made it possible to determine that other citrus species, not exhibiting the bark symptoms, may be carriers of the virus. Many of these carriers have considerably more tolerance to the presence of the virus than the varieties showing bark symptoms, such as sweet orange, grapefruit, and tangerine. The transmission of psorosis by budding to various species, varieties, and hybrids of citrus has been obtained in numerous tests. Knowledge of leaf symptoms, as well as bark symptoms, is being applied in commercial practice to avoid transmission in budding by the employment of virus-free sources for bud wood for the propagation of trees for new plantings.

Types and Symptoms of Psorosis and Psorosis-like Diseases of Citrus. H. S. FAWCETT and L. J. KLOTZ. Two types, psorosis *A* and psorosis B, have similar bark symptoms, the latter with more rapid spread and with gum formation, often much ahead of the bark eruptions. Leaf symptoms in the form of small, light areas about 1 to 3 mm. on young, rapidly growing leaves are similar in the *A* and *B* types but in older, mature leaves the *B* type may show large chlorotic ring spots approaching those of zonate chlorosis in Brazil, while the *A* type shows only occasional small circular or semicircular ring spots. Symptoms on fruit are rare in the *A* type but in the *B* type are often large, discolored circular to semicircular rings or grooves, the sunken tissue varying from pale yellow to brown, with occasional necrotic breakdown. In fruits of grapefruit many irregularities often may result in bumpy, misshapen fruit. Concave gum disease has concavities in the bark, with gum-infiltrated layers in the wood and flecking type of symptoms on young leaves, similar to those of psorosis *A* and *B*. It is possible that *A* and *B* are variations in the effect of the same virus and that the concave gum disease is closely related. The leprosis in Florida was bark symptoms on older twigs, somewhat similar to psorosis, but no definite leaf symptoms in the rapidly growing leaves have been noted. The circular spots on mature leaves and on fruit are distinctly different from those of either psorosis *A* or *B*. The concentric ring spot of South Africa has spots on leaves and fruit, with certain resemblances to some of the spots occurring in leprosis. It is suspected that several of these diseases are due to the same virus or closely related viruses.

Effect of Sodium Citrate on Release of Curly-top Virus from Alcoholic Precipitate of Plant Juice. J. M. FIFE. Sodium citrate was very effective in releasing the curly-top virus from the alcoholic precipitate of sugar-beet leaf juice or tomato-plant juice. Maximum infection was obtained with 6.8 mM. sodium citrate. Where the extractable virus content of the plant juice is low, as shown by the percentage infection obtained with water as the extracting solution, the sodium citrate method of extracting the virus is far superior to the method in which water alone is used. The virus is not inactivated in the juice at pH values as low as 2.2 because infection was obtained with sodium citrate but not with water alone.

Studies of Selected Strains of Curly-top Virus. N. J. GIDDINGS. Four curly top-virus strains designated as 1, 2, 3, and 4 have been recognized by the differential reactions of varieties of sugar beet, *Beta vulgaris*. Percentages of plants infected and severity of symptoms were used as bases of comparison. Virus strains 1 and 3 induced severe symptoms in susceptible beets; but strain 3 seldom infected the resistant beet used, while strain 1 infected a high percentage of resistant beets and induced very obvious symptoms. Strains 2 and 4 induced mild symptoms in susceptible beets; strain 4 infected only a small percentage of the resistant beets, while strain 2 infected a high percentage of them but induced very mild symptoms. The highly resistant beet variety, 1167, showed distinctive reactions to each of the 4 virus strains, and the differences were highly significant statistically. Tobacco, *Nicotiana tabacum*, and tomato, *Lycopersicon esculentum*, were not infected by virus strains 2 or 4. Plantain, *Plantago erecta*, peppergrass, *Lepidium nitidum*, and Great Northern variety of bean, *Phaseolus vulgaris* L., were not infected by virus strain 2. Susceptible beets infected with the less virulent strains were not immunized from the more virulent strains but appeared to be rendered more susceptible to severe injury by such prior infection.

A Plea for Boldness. J. LEE HEWITT.

Species of Sclerotinia Causing Brown Rot of Deciduous Fruits in California and Their Distribution. WM. B. HEWITT and L. D. LEACH. Brown rot of deciduous fruits is one of the most important disease-control problems of the California growers of stone fruits. It has been essentially one of blossom and twig blighting with an occasional year of fruit rotting, and has occurred mostly in the coastal regions in apricots, almonds, cherries, and prunes. In the past few years, however, large amounts of brown rotting of peach fruits have occurred in the peach-producing areas of the interior valleys.

On March 3, 1936, apothecia of *Sclerotinia fructicola* were found developing from mummied peach fruits in the peach orchards of the upper Sacramento Valley, apparently the first time they have been found or reported in the State.

Sclerotinia fructicola was compared with cultures isolated from blighted apricot twigs. The 2 cultures were different in gross culture characteristics, and the latter was identified as *S. laxa* Adhr. and Ruh.

A survey of the deciduous-fruit districts of California was made for fungi causing brown-rot blossom and twig blight and fruit rotting. In so far as the survey has been made, only 2 species, *Sclerotinia fructicola* and *S. laxa* have been found.

Inheritance in Cucumis melo of Resistance to Powdery Mildew (Erysiphe cichoracearum). I. C. JAGGER, T. W. WHITAKER and D. R. PORTER. Powdery Mildew Resistant Cantaloupe No. 45, now the leading variety in the Imperial Valley of California, was developed from a cross between the very susceptible variety Hales Best and a highly resistant plant selected from an unfixed variety from India. This resistance seems to be inherited as a simple Mendelian dominant factor. In the F_1 generation from crosses between homozygous resistant and homozygous susceptible plants, all plants show the same high degree of resistance as the resistant parent. In a series of records on the F_2 generation, the resistant plants totaled 233 and the susceptible 86. This approaches the expected 3-to-1 ratio. Back crosses between F_1 plants and the susceptible parent gave 21 resistant to 25 susceptible plants, which is within the range of expectation for a 1-to-1 ratio.

These data are based on records made in Imperial Valley during the early part of the season of 1938 and in preceding seasons. In midseason of 1938 a new biologic form of the mildew appeared in that section, to which the Resistant No. 45 variety shows, at least, considerably susceptibility. No data are available on resistance to this new form.

The Nature of Water Damage to Citrus Fruits. L. J. KLOTZ. Water injury to citrus fruits is important mainly on navel oranges, which mature during the rainy season. Experimentally, however, any of the common species and varieties of citrus can be damaged. The first evidence of injury is the appearance of minute cracks or checks in the cuticle and one or more layers of underlying cells in the vicinity of the navel convolution, wounds or shoulder. Fresh wounds in the rind such as those caused by thorn and twig punctures and abrasions, sand, hail, and wind or injury by such agencies as frost and oil sprays are important in increasing the incidence of water spot. Old calloused or healed rind scars, as those initiated by thrips, tortrix, katydids, rubs, scratches, and chemical burns during the period of rapid growth of the young fruit do not affect the incidence of water spot. The cracking or checking stage is followed by a rapid entrance of external water evidenced by a water soaked, blistered-like area, which is readily invaded by blue and green molds and decayed. The water taken up by the hydrophilic albedo cells affects the permeability of the cells surrounding the oil glands and the toxic oil is liberated into the albedo. If dry weather follows the initial stages of the trouble the tissues dry down leaving an ugly brown, collapsed area that becomes covered by a dark *Cladosporium* sp. when wet weather recurs. The more mature the fruit the more readily it is damaged, for the rind becomes less elastic and, because of increase in sugars, has a greater osmotic value. Kumquats, which are very readily and severely damaged by rains, have a greater osmotic value and pectin content in the sap of the rind than do navels and Valencias.

Curly-top Virus in Root Tips of Sugar Beets and Beans. C. F. LACKEY. Curly top has been found to be translocated through the phloems of its host plants. Studies made on root tips of sugar beets and beans show that the virus also is found below the protophloem sieve tubes in the meristematic region. A method has been developed by which sections .2 to .5 mm. can be cut from fresh root tips and tested for the presence of virus. These tests show that a much higher percentage of infection on susceptible test beets can be obtained from tissue below the protophloem sieve tubes than from sections including them or from checks consisting of entire rootlets 5 mm. long. When four sections were cut from tips, each .5 mm. long, twice as much infection was produced with virus from the third section than from the second, but less than from the root cap section. Similar results were obtained from diseased bean-root tips, although there was not so much difference in percentage of infection between the 3 lots of tissue. Degeneration of the pericycle of the root tips follows the sieve tubes closely. A very few cases of degeneration of cells in the meristematic region directly below the extreme end of the sieve tubes have been found. Usually there is no visible evidence of the virus in this region of the root tips.

Seed Treatment for the Control of Damping Off of Sugar Beets in Northern California. L. D. LEACH and B. R. HOUSTON. Isolations indicate that soil-borne *Pythium* sp. and *Rhizoctonia solani* are the most important organisms causing damping off of sugar beets in northern California. *Phoma betae* is frequently destructive on seedlings from European grown seed, but has not been observed on seedlings from domestically produced seed. This relation was confirmed by laboratory examination, which showed that certain lots of European seed carried considerable Phoma infection, whereas none was observed on domestic seed. Greenhouse and field trials indicate that cuprous oxide is an effective seed treatment where damping off is caused by *Pythium* sp., but that organic mercury compounds (Ceresan and New Improved Ceresan) are more effective when *Rhizoctonia* and *Phoma* are involved. In severely infested fields the uniformity, as well as the density of thinned stands, has been improved by seed treatment, as in the following example: non-

treated—beets per 100 feet, 67.6, coefficient of variation, 71.4 per cent; treated—beets per 100 feet, 96.7, coefficient of variation, 46.8 per cent.

Pythium Disease of Fibrous-rooted Begonia. J. T. MIDDLETON, C. M. TUCKER, and C. M. TOMPKINS. A disease affecting the crown, basal portion of the stem, and the leaves of fibrous-rooted Begonia plants, variety *Fire Sea*, was observed on bedding plants in Berkeley, California. A similar disease was found on fibrous-rooted Begonia plants, varieties *Carmine, Christmas Cheer*, and *Primadonna*, grown in greenhouses at Columbia, Missouri.

Symptoms of the diseases are identical for the varieties mentioned. In naturally infected plants the crown and lower portion of the stem become somewhat discolored, water-soaked, and soft. Infection may progress up the stem 2 or 3 inches, causing the stem to collapse. In infected leaves the petioles become limp and dark, the laminas water-soaked and flaccid; subsequent abscission of infected leaves is not uncommon. When potted plants were grown in close proximity with one another the disease was quite serious, being distributed throughout the plot by means of leaf infection.

Pythium ultimum was isolated from the Berkeley material. *Pythium de Baryanum, Pythium splendens*, and *Pythium ultimum* were isolated from the Columbia material.

All of the *Pythium* isolates proved pathogenic to the Begonia varieties mentioned by adding the fungus to the soil; each organism induced the disease individually.

Cucurbit Powdery Mildew on Carica Papaya. P. A. MILLER. A powdery mildew attacking seedlings of *Carica papaya* in the greenhouse at Los Angeles was first observed in November, 1935. Cross-inoculation experiments, using conidia from papaya plants on dwarf sunflower plants and conidia from mildewed sunflower plants on healthy papaya seedlings, resulted in no infection. In the summer of 1936, papaya seedlings grown in the greenhouse at Riverside were successfully inoculated using conidia from field-grown cucumber plants. All of the plants inoculated developed sporulating mildew spots on the cotyledons, stems, and leaves. Cucurbit plants in the greenhouse at Los Angeles were inoculated with conidia from these mildewed papaya seedlings. The mildew that developed on the inoculated cucurbit plants spread to the lower leaves of large mature papaya plants growing in the same greenhouse. In October, 1936, the lower leaves of mature papaya plants growing in the open in Orange County, California, were found attacked by cucurbit powdery mildew. Mature leaves and plants are only slightly affected, but young seedlings may be severely injured by the disease. The results of the cross inoculation experiments and field observations indicate that *Carica papaya* is a host of the physiologic race of cucurbit powdery mildew common in this region.

Diseases of Ornamental Plants in Southern California. P. A. MILLER. A pythiacious type of fungus was found to be the cause of a gummosis affecting young *Acacia floribunda* trees in the Santa Monica region. Although the organism has not been definitely identified, inoculations with it have produced typical gummosis lesions. All stages of the root-knot nematode, *Heterodera marioni*, have been recovered from hypertrophied roots of *Tritoma* sp. and *Passiflora mollissima*. Brazilian Pepper, *Schinus terebinthefolius*, trees affected with *Verticillium* hadromycosis were found near Arcadia, California. An extensive planting of *Centaurea, Calendula* and *Ranunculus* plants on the same property were found severely attacked by *Sclerotinia sclerotiorum*. An oil-burning race-track drier, which covered an area 8 × 12 ft., was used to burn over 8 acres of level planting. Four minutes' exposure raised the temperature of the soil 1 in. below the surface to approximately 600° F. Sclerotia on the soil surface were reduced to ashes and those 1 inch below the surface did not germinate after a 4-minute exposure to heat from the burner. The soil of other areas was treated with 1–100 formaldehyde solution at the rate of 1 gal. per square foot after the plants had been removed. About 50 per cent of the sclerotia tested after exposure to this treatment did not germinate.

A Possible Significance of the Blue Nuclei of the Black Tulip. FRANK P. MCWHORTER.

Susceptibility of Cupressus and Allied Species to Crown Gall. CLAYTON O. SMITH. Artificial inoculations with cultures of the crown-gall organism, *Bacterium (Phytomonas) tumefaciens* on different species of the Cupressaceae showed that *Librocedrus decurrens, Thuja orientalis, T. plicata, T. occidentalis* and *Thujopsis dolabrata* produced galls 5–25 mm. in diameter. The following species of *Cupressus* were received as small seedlings (except *C. lusitanica* and *C. sempervirens*) from Rancho Santa Ana Botanic Garden, California: *C. arizonica, C. bakeri, C. duttoni, C. forbesi, C. goveniana, C. macrocarpa, C. macnabiana, C. pygmaea, C. sargenti, C. thurifera, C. lusitanica* SPI 73844, and *C. sempervirens*. Galls 15–25 mm. in diameter developed on the above species of *Cupressus*. The galls on *Cupressus* are at first globose and have a normal bark, but with age (about 1

year), the galls may become rough and then resemble crown gall as it develops on other susceptible hosts. *Juniperus virginiana, J. procera* SPI 60553 are susceptible, while *J. cedrus* SPI 57080 and *J. californica* did not develop galls when inoculated. Inoculations on *Chamaecyparis lawsoniana* gave overgrowths that are not entirely typical of crown gall.

Pink Disease, a Bacteriosis of Pineapple Fruits. C. H. SPIEGELBERG. A pink to brown internal discoloration of ripe pineapples, often with cantaloupe odor, is a bacteriosis, as shown by isolations and inoculations. Faint discoloration beginning near the normal-appearing shell, extends laterally and inwardly, darkening and softening tardily. Heating browns affected tissues and juice; $(NH_4)_2HPO_4$ accelerates and intensifies the discoloration.

Pink disease occurs chiefly from January to March in low-acid fruits, but highly acid fruits are susceptible when inoculated, both in summer and winter.

The pathogen, variable in colony type, pigmentation, aroma, and pathogenicity, has also been isolated from field-collected surface-sterilized: *Calophyllum inophyllum, Citrus aurantifolia, Coffea* sp., *Eugenia malaccensis, Ficus macrophylla, Garcinia xanthochymus, Mangifera indica, Persea americana, Psidium cattleianum, P. guajava,* and *Terminalia catappa* fruits; also adult *Drosophila melanogaster, Carpophilus* spp. and pupal *Ceratitis capitata.* Fed to aseptically reared *Drosophila* larvae, it was reisolated from larvae, pupae, and adults, but these adults failed to infect pineapple. Adult *Ceratitis* females, but not males, infected Anjou pear and tomato, indicating ovipositor inoculation. Results with pineapple, not a host of *Ceratitis*, are inconclusive. The pathogen, associated with *Ceratitis* ovipunctures, is more abundantly distributed in the above 11 fruits than in pineapple, indicating pineapple infections probably follow chance visitation of vector.

A Nursery Blight of Citrus Caused by Phytophthora citrophthora. R. B. STREETS. An investigation was made of the nature of an unusual disease of citrus nursery trees in which the leaves suddenly wilted and died and the stem became blackened in a zone 6 to 12 inches long, just above the bud union. The wood of the sweet seedling-orange scion was definitely discolored, and agar cultures of the wood and inner bark yielded a white, cottony fungus growth. The sour orange rootstock was not affected. The fungus proved indistinguishable in cultural characters and pathogenicity from *Phytophthora citrophthora.* This disease, therefore, was considered an apparently undescribed manifestation of brown-rot gummosis. The nursery trees were being held in a closely covered "lath house," and a rather heavy, wet soil had contributed to high humidity, producing conditions favorable for attack by this fungus. Several hundred trees were rendered worthless, but the non-infected trees were saved by the prompt application of Bordeaux mixture, special care being taken to spray the trunks well.

A Root Rot Disease of Citrus. R. B. STREETS. The sudden wilting and death of a number of trees in a 20-year-old grove of tangerines was investigated by J. G. Brown and the writer. It was found that, while the larger roots and branch roots of trees in intermediate and early stages of the disease appeared healthy, the smaller roots and feeding roots were dead. Cultures of the infected roots yielded a high percentage of colonies of *Fusarium*, of 2, or possibly 3, species, resembling those often found associated with dry root rot of citrus. The pathogenicity of these cultures is being tested and control measures that have yielded favorable results in case of Phymatotrichum root rot are being tried. Cultures made from the feeding roots of grapefruit, navel orange, and Valencia orange from trees showing symptoms resembling early stages of the tangerine disease and resembling our citrus "decline" yielded the same species of *Fusarium.*

Control Measures for Phymatotrichum Root Rot of the Pecan. R. B. STREETS and LLOYD BRINKERHOFF. In the treatment of visibly infected pecan trees, ammonium sulphate alone, apparently satisfactory in many cases, sometimes failed to permanently check root rot under the most severe conditions. Ammonium phosphate (16–20) appeared definitely more promising, and will be tested extensively during the coming season. Sulphuric acid solutions showed definite promise when injected into the soil to a depth of 4 feet, either alone or in combination with ammonium sulphate or ammonium phosphate. The immediate effect is of much value in treating visibly infected trees. Sulphur alone, or in combination, injected or mixed with the surface soil, gives similar but much slower response because of the 3 to 6 weeks necessary for oxidation. Its value, therefore, is preventive rather than curative.

Tree-by-tree mapping of 5 large infected groves totalling 590 acres, and remapping after 5 and 12 months, respectively, demonstrated clearly that it is practical to detect trees slightly infected with root rot long before the symptoms become apparent to the untrained observer. This has made possible the treatment of trees in the first rather than the last stages of the disease with correspondingly greater success.

Effects of Soil Treatments for the Control of Phymatotrichum Root Rot on the Soil and the Pecan. R. B. STREETS and LLOYD BRINKERHOFF. Over 600 soil samples from treated and nontreated soils were analyzed for total soluble salts, total nitrogen, nitrate nitrogen, phosphates, sulphates, and pH. Increases in nitrate nitrogen, total nitrogen, phosphates, and sulphates are much greater in the surface foot and second foot and progressively less in the third and fourth feet.

Applications of ammonium phosphate reduced the pH more than equivalent amounts of ammonium sulphate, but the principle toxic effect lies in the ammonium ion. Sulphur and sulphuric acid treatments probably depend upon the reduction of the pH, although the response of trees has exceeded that expected from changes in pH.

The pecan, at least in the soils tested, shows very great tolerance to the chemicals used for root-rot control, and the danger of injury by the usual dosages appears to be very slight. No injury was produced by less than 12 times the standard application (1 lb. to 10 sq. ft.) of ammonium sulphate applied as a single treatment, by less than 10 times the standard application of ammonium phosphate 16–20, or less than 3 gal. per sq. ft. of a 7 per cent (by volume) sulphuric acid solution.

Acquired Tolerance of Curly Top in Nicotiana Tabaccum. J. M. WALLACE. *Nicotiana tabaccum* plants commonly recover from curly top. Plants showing severe symptoms on terminal leaves frequently produce basal or lateral shoots entirely free of symptoms or showing only inconspicuous symptoms. In other instances recovery comes about in the production of less severely diseased leaves and tissues in the terminal shoots that have shown severe symptoms and retarded growth for a period of time. Cuttings grown from recovered plants have an acquired tolerance to curly top as evidenced by their resistance to reinoculation. Cuttings from healthy, previously noninoculated plants are easily infected and develop severe symptoms. Tolerance developed following infection with one strain of curly-top virus protected against inoculation with a second strain of virus. Whether or not the second strain became established in the plants has not yet been determined.

DERIVATIVES FROM AN UNUSUAL STRAIN OF TOBACCO-MOSAIC VIRUS

I. P. NORVAL[1]

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In recent years it has been recognized (3, 5, 6, 7, 9, 12) that the plant viruses often give rise spontaneously to new forms. These forms or strains usually preserve their identity in succeeding host passages and, like the parent virus, occasionally mutate to give still other strains, which can be distinguished from them.

Jensen (7) obtained from the common tobacco mosaic an unusual variant, J–14[2], which is only slightly infectious in Turkish tobacco, *Nicotiana tabacum* L. and tomato, *Lycopersicum esculentum* Mill., causes spreading necrotic lesions, and never becomes systemic. It seemed likely that a study of a large number of variants from strain J–14 might be of value in enabling us to understand more about the "genetics" of the tobacco-mosaic virus group. The purpose of this paper is to present the results of such a study.

MATERIALS AND METHODS

Since the isolation of strain J–14 by Jensen, the virus had been maintained by serial passage in tobacco. Inoculations from a stem lesion were made to the leaves of healthy tobacco plants. A few necrotic primary lesions resulted. Three serial transfers were made to tobacco from single necrotic lesions of this stock. Plants of the first passage were held for about 20 days and in no case showed signs of a second virus. This was considered sufficient evidence that the virus was free from contamination.

It is so difficult to obtain infection with J–14 that carborundum (No. 320) was always either dusted on the leaves or added directly to the inoculum. For transferring from single lesions on leaves Holmes' (4) brass-tube method was employed. At the beginning of the investigation (Table 1) 2–4 plants usually were inoculated from a single lesion. Later (Tables 2, 3, and 4) it was found more convenient to crush several leaf lesions, or a single stem lesion, in a mortar and to inoculate from this with a cloth-covered glass spatula. In this way more uniform infection could be obtained with an average of about 3 lesions per plant. The hand which supported the leaf to be inoculated was covered with a piece of waxed paper. Instruments were sterilized by boiling or autoclaving.

Since J–14 spreads slowly, it was presumed that almost all mutants arising from it would be faster-moving and would probably be able to cause systemic infections on the upper leaves of the plants. From these leaves,

[1] Commonwealth Fund Fellow.

[2] The initials J, K and N have been used to denote strains of the virus isolated by Jensen, Kunkel and the writer, respectively.

TABLE 1.—*Variants isolated from tobacco-mosaic virus, strain J–14, in Turkish tobacco plants infected with virus from single lesions*

Source of inoculum	No. of plants infected	Mutations: Strain number	Mutations: Type in Turkish tobacco	Days to appearance
L[a]	14			
L	12	1	Local, yellow	6
L	13			
S	8			
L	6	14	Local, yellow	5
L	9	18	Local, yellow	
		19	Local, yellow	
L	63	2	Systemic, necrotic	10
		4	Fast-moving, yellow	8
		5	Fast-moving, yellow-green	10
S	12	7	Fast moving, yellow	10
		6	Distorting, green	
L	65	12	Fast-moving, yellow	10
		13	Local, yellow	10
		10	Non-distorting, green	10
		15	Non-distorting, green	20 ±
		17	Distorting, green	20 ±

[a] L indicates inoculum was from a leaf lesion; S that it was from a stem lesion in a Turkish tobacco plant.

TABLE 2.—*Variants isolated from tobacco-mosaic virus, strain J–14, in Turkish tobacco plants infected with virus from several different local lesions*

Source of inoculum	No. of plants infected	Mutations: Strain number	Mutations: Type in Turkish tobacco	Days to appearance
S	6			
L	9			
L	32	41	Severe, yellow	12 ±
		46	Severe, yellow	
		44	Fast-moving, yellow	10
		53	Distorting, green	30
L	39	49	Fast-moving, yellow	6
		50	Slow-moving, yellow	10
L	24	52	Fast-moving, yellow	9
L	3	55	Distorting, green	8
L	23	59	Distorting, green	32
		60	Distorting, green	34
L	35	57	Local, yellow	7
		58	Distorting, green	17
S	15			
S	6			
L	23	68	Fast-moving, yellow	6
		82	Non-distorting, green	6
L	20	94	Distorting, green	36
S	10	87	Non-distorting, green	15
		88	Slow-moving, yellow	

TABLE 3.—*Variants obtained from old stem lesions in Turkish tobacco*

Date	Source of inoculum	No. of plants infected	Mutations: Strain number	Mutations: Type in Turkish tobacco	Days to appearance
Feb. 11, 1938	S	15	90	Distorting, green	5
			92	Local, yellow	7
			93	Non distorting, green	7
			97	Fast moving, yellow	12
Feb. 14, "	S	10	96	Fast moving, yellow	5
			98	Fast moving, yellow	7
			99	Distorting, green	9
Feb. 15, "	S	15	100	Severe, yellow	8
			102	Distorting, green	13
			103	Slow moving, yellow	10
Mar. 8, "	S	15	106	Severe, yellow	8
			107	Slow moving, yellow	7
			126	Fast moving, yellow	8
			108	Distorting, green	5
			112	Severe, yellow	7
			114	Severe, yellow	10
Mar. 9, "	S	20	115	Distorting, green	6
			111	Severe, yellow	6
			116	Fast moving, yellow	10
			117	Distorting, green	7
			118	Local, yellow	6
Mar. 10, "	S	20	119	Fast moving, yellow	6
			120	Local, yellow	6
			121	Fast moving, yellow	8
			122	Local, yellow	8
			123	Slow moving, yellow	9
			124	Severe, yellow	10
			125	Distorting, green	15

TABLE 4.—*Variants isolated from tobacco mosaic virus, strain J–14, in tomato and N. sylvestris*

Source of inoculum	No. of plants infected	Mutations: Strain number	Mutations: Type in Turkish tobacco	Days to appearance
L from tobacco	2			
S from tomato	6			
L from tobacco	15			
L from tomato	14			
S from tobacco	6	62	Fast moving, yellow	24
S from tomato	40	80	Systemic, necrotic	16
		69	Fast-moving, yellow	12
		79	Distorting, green	15
		63	Fast moving, yellow	8
Nicotiana sylvestris				
L from tobacco	34	83	Local, yellow	7
		84	Non distorting, green	9
L from *N. sylvestris*	10 (tobacco)	95	Local, yellow	6
		101	Non-distorting, green	10

it should be possible to remove them uncontaminated with the parent strain. This method was employed throughout the investigation for obtaining variants. Rarely did more than one variant appear in the same plant. However, in view of the possibility that more than one might arise, the mutants were always taken first to *Nicotiana glutinosa* L. and thence by single-lesion inoculation back to tobacco (5).

Mutations arising at the edge of a necrotic lesion might conceivably be unable to move away from the lesion but might be segregated if inoculations were made from the crushed-up lesion. If such a mutant were present in considerable quantity it might appear in all the inoculated plants. Instead of using large sets of plants infected from one source of inoculum, many small sets of plants were, therefore, used and frequent transfers were made from set to set. When, as often happened, a number of systemic strains closely resembling each other appeared in the same set, no attempt was made to distinguish between them, the presumption being that they arose in the parent lesion as a single mutation and did not move away. Each variant was assigned a number when it was first seen. Subsequently, if several variants arose in the same set and appeared to be identical, only one was retained. For this reason the numerals designating the strains in the text are higher than the number of strains isolated.

Except where otherwise stated, the experiments were carried out during the autumn and winter months of 1936 and 1937 in a greenhouse held at about 75° F. and kept comparatively free from insects by regular fumigation.

RESULTS

Sixty-two variants were isolated from strain J–14 by passage to Turkish tobacco, tomato and *Nicotiana sylvestris* Speg. and Comes plants. The data concerning their isolation are summarised in tables 1, 2, 3 and 4. When leaf lesions were used as the source of inoculum about 7 per cent of the infected plants produced variants. In table 3, where the source of inoculum was old stem lesions, about 30 per cent of the inoculated plants contained variants. Most of the variants were visible within 12 days of inoculation, often as primary lesions on the inoculated leaves. It is probable that they arose in the parent lesions but were unable to move away and consequently were not detected. The derivatives can be roughly classified by their symptoms on Turkish tobacco plants.

A. Those not becoming systemic.
   1) Local yellow type—N1, 13, 14, 18, 19, 43, 57, 83, 92, 95, 118, 120, 122

B. Those becoming systemic.
   1) Slow-moving yellow type—N50, 88, 103, 107, 123
   2) Fast-moving yellow type—N4, 5, 7, 8, 44, 49, 52, 56, 96, 97, 98, 116, 119, 121, 126
   3) Fast-moving severe yellow type—N41, 46, 100, 106, 111, 112, 114, 124

4) Non-distorting green type—N10, 16, 17, 93, 101
5) Distorting green type—N6, 15, 54, 55, 59, 60, 85, 89, 90, 94, 108, 115, 117, 125
6) Necrotic type—N2, 80.

The most noticeable characteristic of these derivatives is their wide divergence as to type. They cover a range similar to that exhibited by Jensen's (7) derivatives from tobacco-mosaic virus No. 1, exclusive of the green mosaics. His method of experimentation would necessarily exclude these. There is a high proportion of yellow mosaics that do not become systemic and are difficult to transmit. They differ only slightly from J–14, causing yellowing in tobacco, which is often followed in a few days by necrosis of the yellowed lesion. No special emphasis should, however, be attached to their close resemblance to strain J–14, since the green-mosaic viruses also frequently give rise to mutants of the localized yellow type.

The slow-moving, yellow strains N50, 103, and 107, are very similar to Jensen's (7) virus No. 111, which he described as a moderately slow-moving, yellow-spotting strain in tobacco. They do not cause vein-clearing symptoms nor a typical mottle. Secondary lesions show as isolated yellow spots with green centers or as a yellowing that spreads out from the veins. The strains are not highly infectious. Strain N88, which is distinctly different from the other members of the group, will be discussed later.

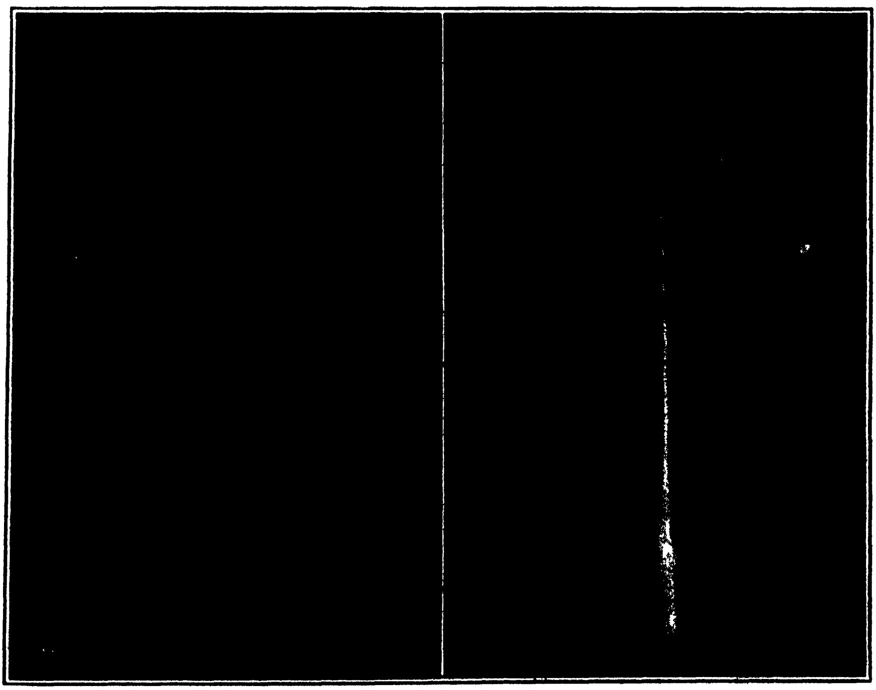

FIG. 1. Symptoms produced in tobacco by strain N4. A. Twenty days after inoculation. The plant is in the "recovery" stage, and subsequent invasion of the young leaves is beginning. B. One of these leaves, 15 days later, illustrating the formation of irregular yellow and green rings. (Photograph by J. A. Carlile.)

The fast-moving yellow types can be conveniently divided into classes on the basis of their severity on tobacco and other hosts. The less severe type shows a yellow mottle with some distortion of the leaves and a tendency to form yellow and green rings on tobacco (Fig. 1, B). There is little, if any, necrosis of the yellowed leaves. It has been possible to separate some of the viruses of this group on the basis of their symptoms in tomato and *Nicotiana sylvestris*, while others are so closely similar that it has not been possible to distinguish between them.

The severe, yellow-type strains cause first an intense yellowing of the leaf, which is followed rapidly by necrosis of the tissue (Fig. 2, C). If tobacco plants 4–6 inches high are used, the stems die usually near the base, and the young stunted leaves show a mixture of green, yellow and necrotic tissue. If larger plants are used, stem lesions are slower in appearing and the plants suffer less damage. On tomato the severe mosaics cause primary and secondary necrosis (Fig. 2, A, B), killing young plants within a short time. Jensen's (7) severe yellow mosaic variant, J–14D1, is similar to these strains in its action on tobacco and tomato.

Little attention has been paid to the green-mottling variants beyond dividing them into two groups on the basis of their ability or inability to cause distortion in tobacco.

Necrotic Type of Turkish Tobacco: Strain N2. Since no strain of tobacco-mosaic virus has been previously reported to cause systemic necrosis in Turkish tobacco, the symptoms produced by strain N2 will be briefly described. The symptoms were visible in 10 days in 4 plants of a December 3 set, inoculated from the same parent lesion. Subsequent tests on tobacco, tomato, *Nicotiana sylvestris*, and *N. glutinosa* showed that only one strain was involved. In Turkish tobacco, necrotic primary lesions appeared in 4–5 days (Fig. 3, A). Under some conditions the lesions were at first yellow, but they broke down rapidly. When vigorous plants up to about 6 inches in height were used, the virus almost always became systemic. When older plants were inoculated, the infection frequently remained localized. The onset of systemic infection generally appeared in the form of a few large yellow or necrotic spots, frequently connected with the midrib or with one of the larger veins of the young leaves. Necrosis of the yellow tissue occurred within a few days. In a later stage the most striking effect was necrosis of the veins, especially the midribs, of the young leaves, often with little or no destruction of the contiguous leaf tissue (Fig. 3, B). This resulted in a curious downbending and twisting of the top leaves and was characteristic of the disease. Stem lesions occurred in plants diseased for about 3 weeks and most commonly developed near the base of the stem. They were much lighter in color than those produced by strain J–14.

In *Nicotiana sylvestris*, strain N2 caused primary necrotic lesions in 5 days. The virus did not always become systemic, especially if mature leaves were inoculated. When systemic invasion occurred, the veins and particularly the midribs were killed.

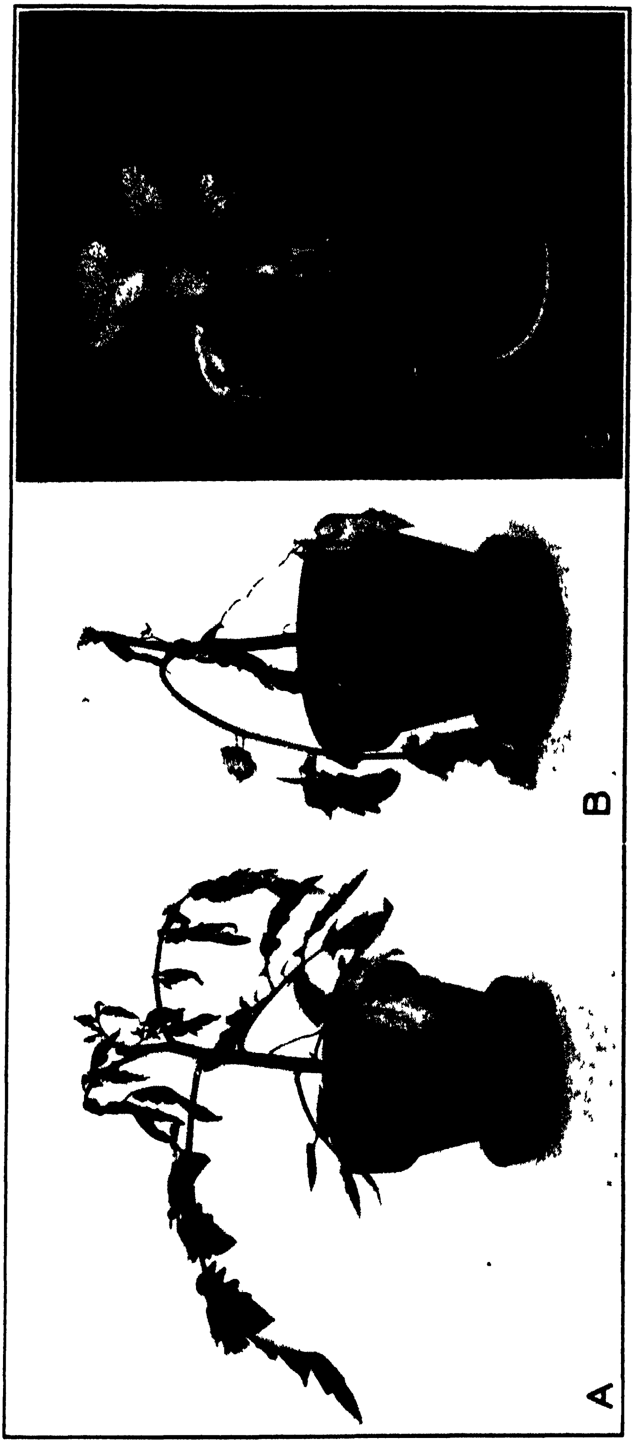

FIG. 2. Symptoms produced by the severe, yellow-type strains in tomato and tobacco. A. Strain K13 in tomato 12 days after inoculation. B. Strain N41 in tomato 14 days after inoculation. C. Strain N41 in tobacco 12 days after inoculation. (Photograph by J. A. Carlile.)

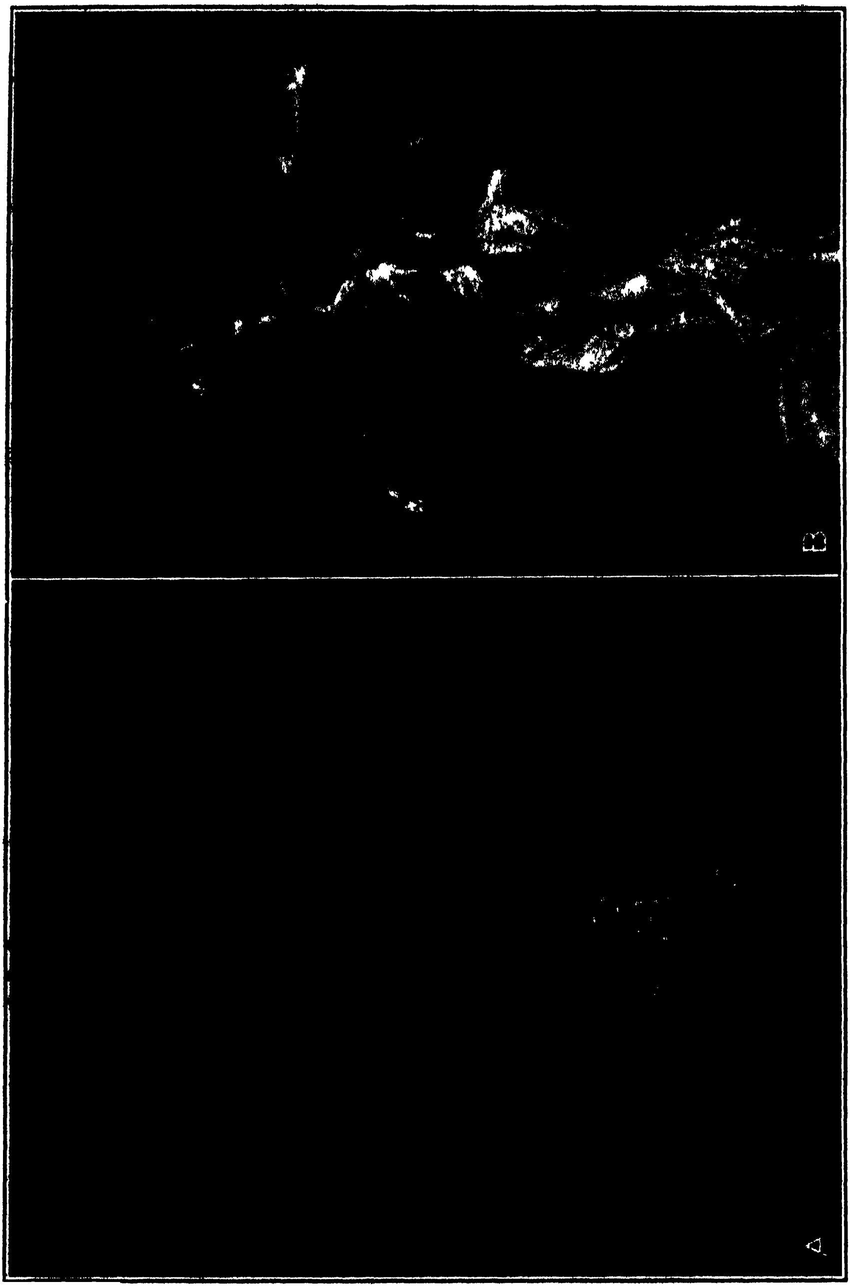

FIG. 3. The primary (A) and secondary (B) symptoms produced in tobacco by strain N2. Note in (B) necrosis of midribs of some of the upper leaves. (Photograph by J. A. Carlile.)

In tomato the first signs of infection were primary necrotic lesions, followed in a few days by secondary leaf spotting and petiole flecking. Within about 2 weeks the young leaves, the inoculated leaf and the large leaves on the same side as the inoculated one, became completely necrotic. Stem lesions did not invariably occur, but, when they did, the stem below the cotyledonary leaves was often killed. The growing-point seldom was destroyed, as usually happened after infection with Jensen's derivative, J-14D1. Otherwise, its action on tomato was similar.

Comparison of Mutants. In the process of maintaining strain J-14 over a period of about 18 months, the virus was transferred serially to a considerable number of tobacco plants by Dr. Kunkel. The plants were held in an actively growing condition for long periods of time. Some developed systemic infections of various types. The variants isolated from such infections were made available for comparison with those obtained in this work.

From their symptoms on tobacco this second group of mutants can conveniently be divided into a number of classes:

A. Those not becoming systemic
   1) Yellow type—none

B. Those becoming systemic
   1) Slow-moving yellow type—K16
   2) Fast-moving yellow type—K1, 5, 9, 11, 12, and 14
   3) Fast-moving severe yellow type—K17, 13
   4) Nondistorting green type—K10
   5) Distorting green type—K2, 3, 4, 6, 7, 8, and 18
   6) Greenish-yellow type forming necrotic rings—K15

The first obvious difference between the two sets is the absence of the local, yellow type in the second set. In maintaining J-14, nonsystemic derivatives were not looked for and, thus, only systemic variants were collected. In the first set the local, yellow derivatives appeared about 6 days after inoculation and the presumption was that they were already present in the inoculum. If the necrotic lesions of J-14 occur near the base of the leaf, it seems unlikely that these local yellow mutants can enter the stem and reach the leaf above. It is possible that some of the mutations recorded were not derived directly from J-14 but from a slow-moving mutant of J-14.

The systemic strains, apart from K15, fall naturally into the same types as the strains subsequently isolated by the writer. The slow-moving yellow strain, K16, looks very like the strains N50, N103, and N107 in tobacco. On mature leaves of tomato they all produce concentric rings of yellow and green tissue. Figure 4 illustrates the symptoms produced by strain K16 on the inoculated leaf of a tomato plant.

Among the fast-moving yellow types many strains of the two sets have been indistinguishable from each other by their symptoms on tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa* plants growing at the ordinary

FIG. 4. Concentric rings of yellow and green tissue produced on the inoculated leaf of tomato by K16, one of the slow moving, yellow type strains. (Photograph by J. A. Carlile.)

greenhouse temperature (75° F.). During the summer, when the greenhouse temperature (13) in the daytime was frequently above 90° F., the following strains could not be differentiated: K1, K5, K9, K11, K12, K14, N8, and N4. Of the severe fast-moving strains, K13 looks identical on tobacco and tomato plants with several of the severe strains that were subsequently isolated.

The strain that forms necrotic rings in tobacco finds no parallel in the first set and will be described briefly. The disease it produces resembles closely that described by Johnson (8) as "ring mosaic."

Necrotic-ring Type: Strain K15. On Turkish tobacco greenish-yellow, primary lesions appeared 4 days after inoculation. Each lesion then de-

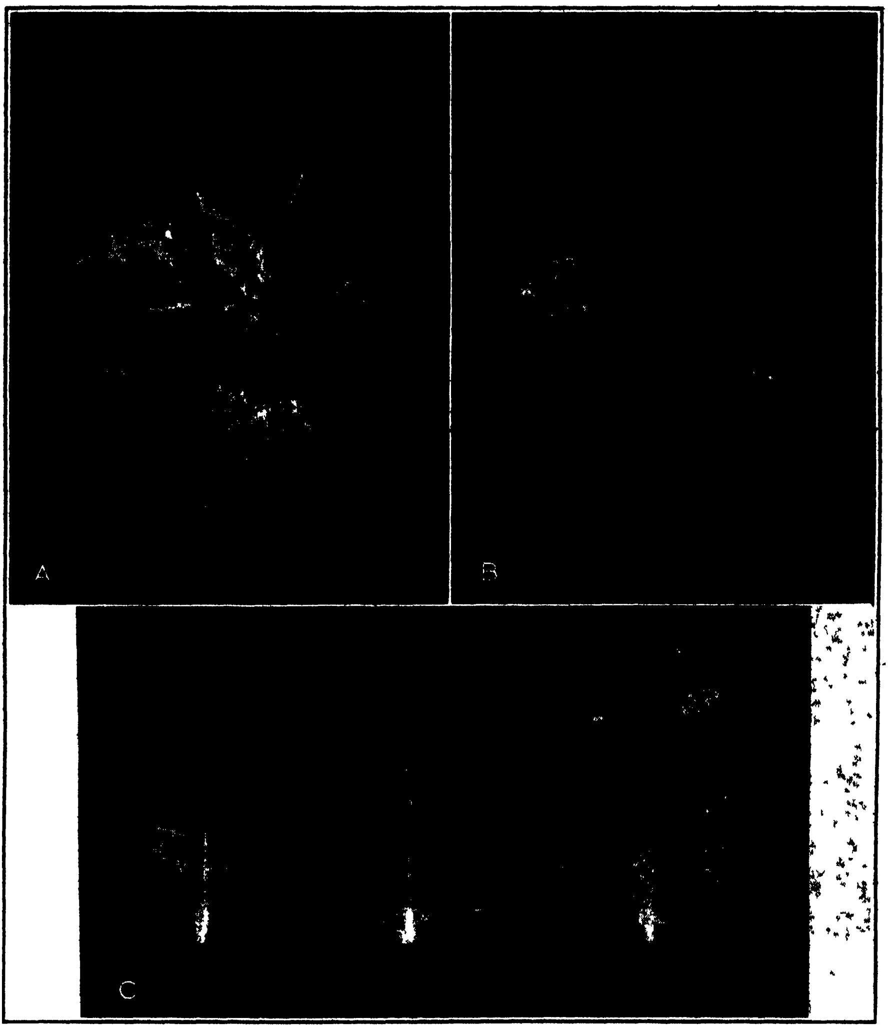

FIG. 5. Symptoms produced by strain K15 in tobacco. A. Primary lesions with necrotic rings on the lower leaves. B. Secondary necrotic spotting. C. Stages in the formation of necrotic rings. (Photograph by J. A. Carlile.)

veloped a ring of dead tissue at its periphery (Fig. 5, A). In some cases a necrotic center as well as margin was evident. Eight days after inoculation the whole lesion became necrotic. In other cases necrotic lesions were the first signs of infection.

Systemic infection appeared first as a clearing-of-veins followed later by a greenish-yellow mottle on the young leaves. On the leaves already fully developed, infection might follow the veins to give oak-leaf patterns of yellowed tissue edged with necrotic, or, when infection occurred in isolated spots, each large spot might at first be surrounded by a ring of necrotic tissue before the lesion dried out (Fig. 5, B).

Necrosis of the young infected tissues started some days after the mottle when the leaves began to expand. In general, the necrosis began at the edge of the yellowed tissue and spread inwards. Sometimes it formed irregular rings; at other times it was a wavy line only a few millimeters long (Fig. 5, C). As the plant grew, the lower leaves shriveled up and died, while the size of the upper leaves was greatly reduced. Stem lesions usually did not occur.

Nicotiana sylvestris was not highly susceptible to infection with K15. In 5 days large greenish-yellow lesions with necrotic edges appeared. These lesions rapidly became completely necrotic. Systemic symptoms appeared as a necrotic spotting connected with the veins of the young leaves. Initially the necrosis might assume a ring form. The plants were killed in about 30 days.

On tomato the symptom picture was much like that described for N2.

Infectivity. It was noticed that the different yellow strains varied greatly in their infectivity. There seemed to be a correlation between the rapidity of movement in tobacco and tomato on the one hand and infectivity on the other. For example, the local, yellow-type strains have a low infectivity; the slow-moving yellow strains seemed to be intermediate in infectivity between the local, yellow strains and the fast-moving, yellow strains. An experiment was run to check this observation, using a typical strain from each class. As the strains formed distinct, yellow lesions in tobacco, it was used as the test plant. Each dilution of virus was inoculated by rubbing with a sterile pad to 5 leaves of each of 2 tobacco plants. The lesions were counted on the fifth day. The results are recorded in the graph (Fig. 6). Both strains that become systemic were very much more infectious than the local, yellow strain. The fast-moving, yellow strain was about 10 times as infectious as the slow-moving, yellow strain. No adequate explanation for the correlation between infectivity and rate of movement has been found.

Frequency of Origin. Table 5 shows the frequency with which strains of the different types have been isolated. It can be said that, in comparison with the other strains, the slow-moving, yellow-type and the nondistorting green strains arise infrequently. Since the local, yellow-type strains are much less infectious than any of the other types, it is presumed that they

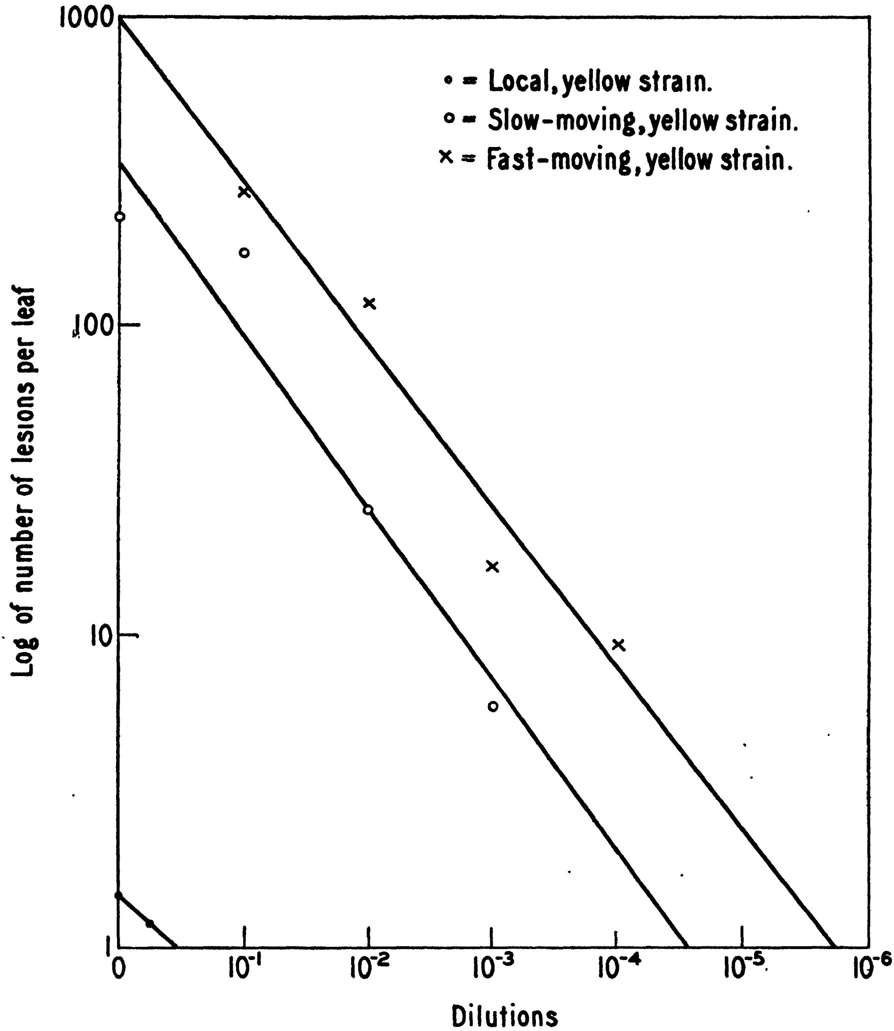

FIG. 6. Comparison of infectivity of three yellow strains that differ in the rates at which they move in the tobacco plant.

were more difficult to isolate. In view of this the large number obtained probably means that they were the most frequent type arising. The high

TABLE 5.—*Frequency of isolation of strains of the various classes*

Strain type	Number of isolates		Total number of isolates
	N	K	
Local, yellow	13		13
Slow-moving, yellow	5	1	6
Fast-moving, yellow	15	6	21
Severe, systemic	10	3	13
Non-distorting, green	5	1	6
Distorting, green	14	7	21

proportion of severe, systemic strains that cause necrosis of leaf and stem in tomato is interesting because of the severity of the parent strain and the fact that Jensen obtained none of them among the strains isolated from the ordinary tobacco-mosaic virus. The fast-moving yellow and the distorting, green strains are comparable in infectivity and were obtained in about equal numbers. Consequently, it is thought that they arose with about the same frequency.

FIG. 7. Symptoms produced in tobacco by strain N88. (Photograph by J. A. Carlile.)

From the point of view of the frequency of origin, strain N88 is exceptionally interesting. In common with strain J104 it produced a mild, yellow spotting in tobacco (Fig. 7), was not highly infectious, and did not move readily. In *Nicotiana glutinosa* both strains produced minute, necrotic lesions that later developed a yellow halo. Jensen (7, p. 78) figures the lesions caused by strain J104 on *N. glutinosa* leaves. From the large number of variants isolated from the common tobacco-mosaic virus and its

derivatives, only two have been reported to form the atypical minute, local lesions in *N. glutinosa*.

DISCUSSION

In this study it has been observed that most of the variants arose a short time (*ca.* 10 days) after inoculation and could first be seen as primary lesions upon the rubbed leaves. That variants arose early, and frequently were visible on the inoculated leaf, means either that they were already present in the inoculum or that they were introduced as contaminants during transmisison. The evidence, accumulated during this investigation, from check plants and from a thorough testing of the methods of transmission, renders it highly improbable that the variants represent contaminations. Further evidence that they are mutants is furnished by the character of the strains themselves. For example, the necrotic and some of the yellow-type strains are seldom found in nature and are thus unlikely to be contaminants, though some of the highly infectious green or yellow mosaics might be. Also, it has been established that the strains isolated are all related to the tobacco-mosaic virus group. On *Nicotiana glutinosa* none, except strain N88, failed to respond with the production of characteristic necrotic lesions. The following strains: N2, 4, 7, 9, 12, 50, 88 and K9, 12, 13, 14, and 15 all reacted positively when tested serologically against tobacco-mosaic virus serum. The author is indebted to Dr. L. M. Black for carrying out the tests. Strain J–14 and the local yellow-type strains could not be recovered from dried leaf material, but they produced the characteristic local lesions in *N. glutinosa,* and no infection was obtained when they were inoculated to plants already infected with the ordinary tobacco mosaic, so that they are undoubtedly strains of the tobacco-mosaic virus (2, 10).

As a class, the mutants represent a wide range of strains varying in their infectivity, their ability to move, their action on the chlorophyll mechanism and the destruction of tissues. A few significant results emerge from this study. The ability to cause tissue destruction in tobacco and tomato is not necessarily connected with low infectivity and inability to become systemic. For example, N2 and N80 cause primary and secondary necrosis in tobacco. Both can be transmitted readily without the aid of carborundum, and infectivity is still retained by N2 at a dilution of 1: 1000. In tomato N2, 80, 41, 46, 100, 106, 111, 112, 114, 124, and K13 and 15 all cause secondary necrosis and are highly infectious. On the other hand, infectivity, in the cases observed, is certainly correlated with the ability to move out of the inoculated (7) leaf when tested on tobacco or tomato. The yellow type that does not become systemic, and J–14 itself, have an extremely low infectivity, while the slow-moving, yellow type is intermediate in infectivity between the local and the fast-moving, yellow types. No exceptions to this rule have been reported in the tobacco-mosaic virus group.

Another point of interest is the comparatively large number of mutants that are extremely severe on tobacco and tomato, and, in this respect, resemble J–14 but differ from the mutants derived by Jensen (5, 6, 7) from the common tobacco-mosaic virus. Holmes (3) had previously shown a similar relationship between strains of the tobacco-mosaic virus group and their derivatives. He collected yellow mutants from a "masked" and a "distorting" strain. The "masked" strain was conspicuously less able than the "distorting" to invade the young tissues of Turkish tobacco. In all the derivatives of the "masked" strain this character was reproduced, but it was absent from most of the derivatives of the "distorting" strain. Despite the definite tendency of the derivatives to resemble the parents closely in this character, the variants as a whole differed widely from each other and from the parent strain. This is equally true of the derivatives from J–14.

From the reaction of certain classes of derivatives from J–14 in Turkish tobacco, tomato and *Nicotiana sylvestris* the following table has been constructed:

Strain type	Turkish tobacco	Tomato	*N. sylvestris*
A (N2)	Necrosis	Necrosis	Necrosis
B (N41)	Yellow	Necrosis	Necrosis
C (N5, 49)	Yellow	Yellow	Necrosis
D (N4, 7)	Yellow	Yellow	Yellow

It is apparent that there is a progressive increase of tissue sensitivity to strains of tobacco-mosaic virus from Turkish tobacco through tomato to *N. sylvestris*. Strains (type A) that caused necrosis in Turkish tobacco never failed to kill tomato and *N. sylvestris* tissue. Strains (type B) that destroyed tomato tissue always caused necrosis in *N. sylvestris*, while strains (type C, represented by N5, 9 and 49) that were comparatively mild in tobacco and tomato still destroyed *N. sylvestris* tissue. There is a fourth type, D, in which the strains do not cause necrosis in any of the hosts. Schematically, this progressive diminution in severity of the different virus strains may be represented as follows:

$$A = B + = C \genfrac{}{}{0pt}{}{+}{+} = D \genfrac{}{}{0pt}{}{+}{\genfrac{}{}{0pt}{}{+}{+}} \text{ where } + = \text{an ``intensifying'' factor.}$$

Since the host plants are not known to differ from each other in sensitivity to the virus strains by single genetic factors, the "intensifying" factor postulated is not considered to represent a single genetic factor.

Some evidence has accumulated that suggests the possible repeated occurrence of the same mutation. Among the fast-moving, yellow types, certain of the strains obtained at different times in both the K and N series cannot be distinguished from each other by their symptoms on tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa*. From a consideration of the technique and controls, it seems very unlikely that they represent recurrent contaminations. Within the other groups obtained there are many strains

that resemble each other closely. Little attempt has been made to distinguish between them. However, apart from these mutants, the evidence gathered from the systemic yellow-mosaic types tends to show, at least within the limits to which identity can be established, that the same mutation does occur repeatedly.

It is interesting to note that one of the more severe local, yellow strains, N92, which had previously been passed twice through tobacco by single-lesion transfer, when inoculated to 10 tobacco plants produced about 200 yellow and 2 necrotic lesions. Upon subsequent transfer of the necrotic lesions to tobacco only local, necrotic lesions resulted. These lesions were identical with those produced by J–14 in tobacco and the strain could not be distinguished from J–14 in other ways. If it is the same strain, this is a case of a reversion.

Bawden and Pirie (1) and Loring (11) have demonstrated that the tobacco-mosaic virus protein is a nucleoprotein. It is known that the hereditary material in plants and animals is concentrated in the nucleus, which contains nucleoproteins. The different heritable factors are carried on the chromosomes at specific loci. A factor may change spontaneously to give a new factor. In this characteristic the virus protein resembles the gene or unit of reproduction in the hereditary mechanism. It is possible that the study of the heritable variations of the virus protein will aid in the final understanding of the structure of the gene.

SUMMARY

Variants from an unusual strain of tobacco-mosaic virus, Jensen's J–14, have been collected and examined. J–14 has a low power of infectivity, produces in Turkish tobacco local necrotic lesions, and never becomes systemic. The derivatives are widely scattered throughout the possible symptom types in tobacco and include local yellow types, slow-moving yellow types, fast-moving yellow and green types of varying severity, systemic necrotic types, and a strain that forms necrotic rings.

Twelve distinct strains cause primary and secondary necrosis in tomato, and thus resemble J–14 in their ability to destroy tomato tissues. Strains of this type occur rarely in derivatives of the green mosaics.

In tobacco and tomato the ability to destroy tissues seems to be independent of the ability to become systemic, but infectivity is correlated with the ability to move.

Many of the mutants, obtained at different times, cannot be distinguished from each other by their symptoms on Turkish tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa*.

The strains that cause necrosis in Turkish tobacco are regarded as having the full complement of factors for necrosis. Strains that destroy the tissues of more sensitive plants but not those of tobacco are thought to lack some of these factors. By using a range of host plants differing in their

sensitivity, it has been possible to recognize within the different strains of virus several factors for necrosis.

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TWIG CANKERS OF ASIATIC CHESTNUTS IN THE EASTERN UNITED STATES

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INTRODUCTION

The Chinese chestnut (*Castanea mollissima* Bl.) was known in the United States to a slight extent before the Civil War and the Japanese chestnut (*C. crenata* Sieb. and Zucc.; syn. *C. japonica* Bl.) was introduced about a decade after its close (14). Subsequently a number of Japanese chestnuts were grown rather successfully in orchards. The Chinese chestnut was introduced into the United States by the Department of Agriculture in 1907 (3). There was a great stimulus to their introduction when it became generally evident that the commercial stands of our valuable American species [*C. dentata* (Marsh.) Bork.] were doomed by chestnut blight. In the past 10 years Asiatic species, which are resistant to chestnut blight, have been introduced for extensive trial in forest and orchard plantings. Bedwell (1) described many of the factors affecting the survival of these Asiatic chestnuts in forest plantations, the most important of which was dieback induced by drought, twig blights, low temperatures, or planting injury. He found various fungi in association with the twig blights and described one of them caused by *Phomopsis* sp. (2).

The writer also has isolated a number of fungi from dying Asiatic chestnuts, but he has confined most of his observations and studies to those found associated with definite cankers. Three of these (*Cryptodiaporthe castanea* (Tul.) Wehmeyer, *Botryosphaeria ribis chromogena* G. and D., and *Diplodia* sp.) have been proved to cause cankers. The discussion presented herein is based on field observations and experiments during the past 6 years and is centered around these three fungi.

SYMPTOMS OF TWIG CANKERS

Because of the similarity in the external symptoms of the three diseases and the inability to distinguish them definitely by macroscopic examination, the description of symptoms applies to all three. However, the description is specifically based on the disease caused by *Cryptodiaporthe castanea*. This is the one most commonly found and has already been reported by the writer[1] as occurring on Asiatic chestnuts in this country.

The cankers or diebacks occur on trunk, limbs, or twigs. In the vicinity of Washington, D. C., the youngest incipient infections are observed in January and February and are manifested as small areas of diseased bark, a shade darker than healthy bark. The causal fungus develops rapidly, kills the bark and the cambium, invades the sapwood, and induces a brown dis-

[1] Fowler, Marvin E. *Cryptodiaporthe castanea* on Asiatic chestnuts. Plant Disease Reporter 22: 69. 1938.

coloration of the host tissue. In early spring when the fungus is actively growing, the discoloration of the sapwood usually extends beyond that of the overlying bark, but later the entire area of the bark overlying this infected tissue discolors and dies.

During a single season the fungus may girdle a small twig, kill it to its junction with a larger branch, and invade that, too. The leaves on girdled branches usually wilt without yellowing and gradually brown and die. The killing occurs most rapidly from February to June, but some killing may occur throughout the year, especially on trees weakened by other factors. If a stem has not been completely encircled by early summer, the formation of callous tissue begins, sometimes causing the bark to split along the edges of the diseased area and a more pronounced canker results (Fig. 1, A). The long axis of the cankers parallels that of the stem. The cankers are sometimes completely healed over during the summer, but, frequently, killing is resumed the following year, causing the cankers to enlarge and often to girdle the stem (Fig. 1, B).

In a nursery at Glenn Dale, Maryland, 125 incipient cankers on Japanese and Chinese chestnuts were selected for the purpose of determining the avenue of entrance of *Cryptodiaporthe castanea*. Of these cankers 85 per cent had small dead twigs in their centers, indicating that the infection had occurred through those twigs or at their bases, 6 per cent had a dead bud in the center of the canker, 8 per cent had developed around pruning wounds, and 1 per cent had a live bud near the center of the canker.

All 3 fungi fruit readily in the dead tissue, and a microscopic examination of these fructifications, or of cultures made from them or from diseased tissue, is necessary in order to distinguish them.

INOCULATIONS

Inoculations were made on healthy, vigorous, potted 2-year-old chestnuts in an effort to determine whether the fungi found associated with the cankers and dieback twigs were parasitic. All the inoculations were made in the spring when the plants were either dormant, with swollen buds, or with small leaves. A number of fungi were tested, but the results are presented only for the three found to be somewhat parasitic and for the comparable check plants. The failure of the other fungi to parasitize may possibly be due to the conditions of the experiment and may not necessarily mean that they are entirely saprophytic under all conditions.

These inoculation experiments were conducted each spring in the pathological greenhouses at Washington, D. C., from 1932 to 1936. *Castanea crenata* and *C. mollissima* were inoculated each year; *C. pumila* Mill., in 1932; and *C. henryi* Rehd. and Wils., in 1936. Several inoculations were made on both the main stem and the lateral branches of each tree. All check trees were treated just like the inoculated ones, except that sterile agar was used in the wounds on some and nothing in those on others.

The stems to be inoculated were first cleaned by swabbing with 95 per

FIG. 1. Typical cankers caused by *Cryptodiaporthe castanea* on Japanese chestnut. A. Old canker with abundant perithecia on dead bark. B. Canker from which the fungus had spread beyond the original callous tissue in the second year and had girdled the stem. Perithecia occur in center of canker and pycnidia on recently killed tissue below. Remains of a small twig can be seen in the center of each canker. Approximately × 1.

cent alcohol, which was immediately burned off, or with 1:1000 mercuric chloride. A small incision was then made through the bark and cambium into the sapwood with a sterile scalpel. Into these wounds was inserted the surface growth of the fungi produced in culture. These were made from diseased tissue and from single conidia of all 3 fungi and from single asci of the *Cryptodiaporthe* and the *Botryosphaeria.* The wounded places on all trees were wrapped with moistened sterile cotton and the plants were placed in an "iceless refrigerator," like that described by Hunt (11), for 24 to 48 hours before being transferred to greenhouse benches. The cotton over the wounds was kept moist for one to two weeks before being removed.

As results of the inoculations did not differ significantly in different years, only the totals are shown in table 1. In general, plants inoculated while dormant were more easily infected by all three fungi than those with swollen buds or small leaves, but they were not always most severely damaged. *Castanea crenata* was more easily infected and more severely damaged by each of the 3 fungi than was *C. mollissima.* The number of inoculations on *C. henryi* and *C. pumila* was not sufficient to permit drawing definite conclusions on the degree of parasitism on them.

All inoculations were examined every few days in order to observe the killing of the tissue immediately around the inoculation wounds before the fungus had spread sufficiently to girdle the twigs. Some twigs were girdled within 2 weeks after being inoculated, while others, especially those of larger diameter, were not girdled for several weeks. After being girdled by the fungus, many seedlings sprouted at the base. On some twigs the fungi killed the tissue adjacent to the wounds, forming incipient cankers that continued to enlarge for several weeks. Callous tissue later formed around many of these affected areas and more pronounced cankers resulted. In many cases the callus covered the wounds. Pycnidia were formed on many lesions induced by inoculations made with cultures from diseased tissue and single conidia of the *Cryptodiaporthe, Botryosphaeria,* and *Diplodia* and from single-ascus cultures of the first two. Microscopic examinations of these pycnidia showed them to be the fructifications of the imperfect stage of the fungi used. Single-conidium cultures were obtained and compared with the cultures from the original specimens. Both the original cultures and those recovered from inoculations were used in subsequent inoculations and again produced the disease. Each of the 3 fungi were recovered from these inoculations and again successfully inoculated into and recovered from *Castanea mollissima* and *C. crenata* trees.

The checks consisted of 162 wounds on 37 *Castanea mollissima* trees, 213 on 50 *C. crenata,* 16 on 4 *C. pumila,* and 39 on 8 *C. henryi.* Some of these trees were dormant, others had swollen buds, and others small leaves. In no case did a canker form around the wound on a check tree. In addition, a number of trees were inoculated with other fungi cultured from dieback twigs. These inoculations might be considered as further checks, as no cankers resulted from them.

TABLE 1.—*Inoculation of Castanea spp. with Cryptodiaporthe, Botryosphaeria, and Diplodia, from 1932 to 1936*

Inoculating fungus, name, and condition of host	Trees inoculated	Trees infected	Inoculations: Number	Inoculations: Percentage infected	Inoculations: Percentage of infections girdling
	Number	*Per cent*			
Cryptodiaporthe castanea					
Castanea crenata—					
Dormant	15	100	57	91	63
With swollen buds	26	88	107	50	51
With small leaves	9	67	34	47	6
Total	50	88	198	61	50
Castanea mollissima—					
Dormant	13	100	52	67	37
With swollen buds	23	52	98	37	39
With small leaves	13	69	51	55	43
Total	49	69	201	49	39
Castanea henryi	4	100	17	100	88
Castanea pumila	6	83	22	64	57
Botryosphaeria ribis chromogena					
Castanea crenata—					
Dormant	5	100	19	100	47
With swollen buds	16	94	73	88	61
With small leaves	2	100	10	60	17
Total	23	96	102	87	55
Castanea mollissima—					
Dormant	3	100	12	100	33
With swollen buds	15	100	72	83	38
With small leaves	6	100	30	80	42
Total	24	100	114	84	39
Castanea henryi	4	100	20	95	68
Castanea pumila	1	100	4	100	50
Diplodia sp.					
Castanea crenata—					
Dormant	6	100	26	85	50
With swollen buds	17	94	85	71	42
With small leaves	7	86	31	55	24
Total	30	93	142	70	40
Castanea mollissima—					
Dormant	4	100	13	69	56
With swollen buds	14	71	63	56	29
With small leaves	12	83	52	67	34
Total	30	80	128	62	34
Castanea henryi	4	50	19	42	50
Castanea pumila	2	100	8	88	29

ISOLATION AND IDENTIFICATION OF CAUSAL ORGANISMS

At first it was the writer's practice to make several tissue isolations from each diseased specimen and to make single-spore isolations of the fungi fruiting on these specimens for comparison. Hundreds of cultures have been made from 1931 to date, and it was found that the causal organisms

were easily isolated from tissue taken under aseptic conditions from the extreme edges of actively growing cankers or from single or mass plantings of spores. The attempts to isolate them from dieback twigs were often hampered by the presence of other fungi. Isolations were made on poured plates of corn-meal, malt, prune, and potato-dextrose agar with equal success.

Cryptodiaporthe castanea (Tul.) Wehmeyer

The fungus found most frequently forms numerous small black raised pustules within the diseased bark from April to May. About 3 weeks after the pustules form, the overlying bark breaks and the pustules become erumpent. Microscopic examinations reveal that this breaking of the bark occurs at approximately the same time as conidia are produced. The conidia are typically 2-cell, slightly unequal, fusoid, straight or slightly curved, hyaline, $10–13 \times 2.5–3.5\ \mu$. Some 1-cell conidia are usually present. This form of the fungus was identified as *Cytodiplospora castaneae* Oud. Perithecia later form in groups near the basal stroma of the imperfect stage in the summer and by midsummer have pushed their beak-like ostioles up through the ectostroma until they are barely erumpent through the bark. The asci are $35–50 \times 6–9\ \mu$ and the ascospores $10–15 \times 2–3\ \mu$. The ascospores are very similar to the conidia, except that they are slightly larger and may be definitely distinguished by the very short hyaline cylindric appendage usually found at each end. This form of the fungus was identified as *Cryptodiaporthe castanea* (Tul.) Wehmeyer [*Diaporthe castanea* (Tul.) Sacc.] and agrees with the description given by Wehmeyer (18). It is distinguished from *Diaporthe* by the absence of blackened zones in the substratum. Cultures made from single conidia, asci, and ascospores were identical in appearance, and, after growing in test tubes for several weeks, produced identical conidia.

Prillieux and Delacroix (13) described and illustrated *Diplodina castaneae* on European chestnut, which has been considered by later workers to be the same as *Cytodiplospora castaneae* described by Oudemans (12). They, however, described 1-septate, $6–7 \times 1–1.5\ \mu$ conidia borne in separate and distinct pycnidia, while Oudemans described 1-septate, $9–12 \times 2\frac{1}{3}–3\ \mu$ conidia produced in compartments arranged in a circle about a parenchymatous axis. The fructifications of the fungus studied by the writer vary, but, in general, agree with Oudemans's description of *Cytodiplospora*. Dufrénoy (8) illustrated *Cytodiplospora castaneae* with 2-cell conidia. Wehmeyer (18) did not describe the pycnidial stage of *Cryptodiaporthe castanea* in detail and made no mention of 2-cell conidia. He listed *Cytispora castanea* Sacc., *Fusicoccum castaneum* Sacc., and *Malacostroma castaneum* (Sacc.) Hohn under its conidial connections. Although Défago (5) stated that he proved the conidial connection of *Cryptodiaporthe castanea* based on single-ascospore and single-conidium cultures, his description and illustrations are of 1-cell conidia only. Grove (10) described the conidia of *Fusicoccum castaneum* Sacc. (*Cytodiplospora castaneae* Oud.) as being

1-septate when fully mature and stated that the likeness of these conidia to the ascospores of the perfect stage was so great that it was not until he had seen the former in large numbers seated upon their sporophores that he was satisfied that they were not escaped ascospores devoid of their row of guttules. This observation by Grove on the morphology is confirmed by the writer. In addition, the writer established the conidial connection by comparing single-ascospore, single-conidium and single-ascus cultures. The short, cylindric, hyaline appendage often observed at each end of mature ascospores has not been observed on conidia, although numerous examinations have been made.

Botryosphaeria ribis chromogena G. and D.

On August 28, 1931, a fungus that appeared to be *Botryosphaeria ribis chromogena* G. and D. was collected on a Japanese chestnut at Arlington Farm, Virginia, and in April, 1934, one that appeared to be *Dothiorella ribis* was collected on Chinese chestnuts at Natchez, Miss. *D. ribis* had previously been collected on dead American chestnut twigs at several localities. Single-ascospore and single-conidium cultures made from the collections on Asiatic chestnuts were identical and both produced conidia. When grown on corn meal in 100-cc. Erlenmeyer flasks, numerous knob-like stromatic bodies were produced and the medium was colored purplish pink. This fungus is a known parasite of currants, producing the disease called currant cane blight, and it has been distinguished from a similar form by its ability to color starch paste purplish pink. The ascospores on chestnut are $15–22 \times 5–7.5\ \mu$ and the conidia, $14–28 \times 5–8\ \mu$ and are both within the range given for this species. Although Shear, Stevens, and Wilcox (15) stated that single perithecia are often found and that the size of the stromata varies with the host and thickness of the bark, both the perithecia and pycnidia found on chestnut occur in groups in a stroma.

As a further means of determining whether the chestnut organism is identical with the currant cane blight fungus, Dr. Neil E. Stevens suggested that inoculations be made on currants susceptible to the currant cane blight. With this fungus 47 inoculations were made on 10 Fay's Prolific and 23 inoculations on 6 Cherry currants. The checks consisted of 36 wounds on 8 currants of the above-named varieties in which sterile agar was inserted. Further checks consisted of 32 wounds on 7 Cherry currants that were inoculated with *Cryptodiaporthe castanea* and 9 wounds on 2 Cherry currants inoculated with the chestnut *Diplodia*. Of the inoculations made with the chestnut *Botryosphaeria* 23 produced lesions on the Fay's Prolific and 14 on the Cherry currants. The *Dothiorella* stage of the fungus appeared on the killed areas. Single-conidium reisolations were made from these fructifications. No lesions were produced by the *Cryptodiaporthe* or *Diplodia* inoculations or on the check plants. This gives additional evidence that the chestnut *Botryosphaeria* is identical with the one on currants. Smith (16) successfully inoculated *Castanea* sp. with *Botryosphaeria ribis chromogena* isolated from citrus and walnut.

Diplodia sp.

In October, 1931, a specimen of Chinese chestnut, on which a species of *Diplodia* was fruiting, was collected at Jackson, Tenn. The black, globoid, erumpent, pycnidia of this fungus are sometimes produced singly, but usually in groups in a stroma in the outer layers of the bark. The conidia are sub-hyaline and 1-cell when first formed, but, upon maturity, many become 2-cell and dark. They are ovoid to elliptic and measure $18–26 \times 11.5–16\ \mu$. Single-conidium cultures produced conidia that agreed in size, color, and shape with those on the host. This fungus has not been specifically identified. On other collections of Asiatic chestnuts a *Diplodia* with conidia of a different size was fruiting. Inoculations made with cultures obtained from this gave negative results.

DISTRIBUTION AND DAMAGE

In the eastern United States, numerous collections have been made of cankers on Asiatic chestnuts, and *Cryptodiaporthe castanea* has been found to be the most commonly associated fungus. In some plantations no other fungus has been found fruiting on the dead tissue in the cankers. This fungus has been collected and identified on *Castanea* spp. from the following localities: Maine—Cape Elizabeth; New Hampshire—Raymond; Massachusetts—Petersham; Connecticut—Stamford; Rhode Island—Providence; New York—Castile; New Jersey—Newfoundland, Phillipsburg, Washington Crossing; Delaware—Mount Cuba; Pennsylvania—Bally, Barto, Erie, Lancaster, Tryonville; Maryland—Annapolis, Beltsville, Bigpool, Fort George G. Meade, Glenn Dale, Port Republic; District of Columbia; Virginia—Abingdon, Arlington, Buena Vista, Drewrys Bluff, Fairfax Station, Gordonsville, Natural Bridge, Warrenton; North Carolina—Andrews, Asheville, Busick, Chapel Hill, Durham, Franklin, Lake Logan, Morganton, Raleigh, Stillhouse Branch, Willets; Georgia—Albany, Jasper, Savannah; Alabama—Moulton, Muscle Shoals, Prattville; Tennessee—Byrdstown, Harriman, Jackson, Waverly; Ohio—Brecksville, Springfield, Wooster; Michigan —Battle Creek; Texas—Kirbyville. These collections include *Castanea mollissima, C. crenata,* and *C. henryi* in numerous localities and *C. sativa* Mill. from the District of Columbia, only.

This wide distribution of the fungus cannot be considered as evidence that the organism is native, as most of the plantations and orchards were established from trees grown in a few recently established nurseries. As the fungus has been found in some of these nurseries, it could have been distributed from them because, during the transplanting season, in early spring, many infections are incipient and could undoubtedly escape the attention of inspectors. However, the fungus was present in 3 widely separated Japanese chestnut orchards planted 30 to 40 years ago at Drewrys Bluff, Fairfax Station, and Gordonsville, Virginia, long before the establishment of the above-mentioned nurseries. Several years ago when the fungus was first observed in these plantations the appearance of the diseased

trees was such as to indicate that it had been present for some time. Furthermore, at that time additional plantings of Asiatic or European chestnuts had not been made within several miles of these orchards. The same fungus is also abundant on two 43-year-old Japanese chestnuts at Barto, Pa., in the vicinity of which no other Asiatic or European chestnuts have more recently been planted. The source of these infections has not been determined. Numerous examinations of twig fungi on native *Castanea* and *Quercus* spp. have not revealed the presence of any that resemble *Cryptodiaporthe castanea.*

This fungus has been known to occur on *Castanea sativa* in Europe for many years. Prillieux and Delacroix (13) observed a canker disease, which they called "Le Javart," on European chestnut in 1893 and named the associated fungus *Diplodina castaneae.* Oudemans (12) described what is apparently the same fungus as *Cytodiplospora castaneae* on stems of European chestnut collected in Holland in 1893. Later, Ducomet (7) studied the "Le Javart" disease and identified the fungus as *Cytodiplospora castaneae* Oud. This disease has been mentioned as occurring in Europe by other workers, including Foëx (9), Day (4), and Dufrénoy (8). Dufrénoy stated that it parasitized the bark of Japanese chestnut, weakened by transplanting or by some unfavorable factor. Grove (10) reported this fungus on dead twigs and branches of European chestnut in England and gave its distribution as France, Holland, Germany, and Italy. More recently, Défago (5) described in detail a disease of young European chestnuts in a nursery and attributed it to *Cryptodiaporthe castanea* (Tul.) Wehmeyer. He proved its parasitism by inoculations and considered the fungus to be capable of developing a canker that is often completely stopped by the formation of callous tissue unless external conditions lower the resistance of the plant.

In most of the Asiatic chestnut plantations examined, *Cryptodiaporthe castanea* had the appearance of an active parasite, but was sometimes stopped completely by the callus formed around and over cankers during the summer months. Sometimes the killing was resumed the following year and the fungus invaded the tissue beyond the callous ring (Fig. 1, B). Although frost or other unfavorable factors may kill small twigs and allow the fungus to enter, there is no doubt that after it has entered the twigs it is able to invade and kill previously uninjured tissue.

A number of plantings of various strains of Asiatic chestnuts have been made on old abandoned fields, where the growing conditions are not yet suitable for hardwoods (2, 6) and, under such conditions, *Cryptodiaporthe castanea* may kill young seedlings to the ground. An attempt has been made to determine the relative effect of this fungus on vigorous and suppressed trees. Very few Asiatic chestnuts have been planted on sites that may be considered as offering optimum growing conditions. In some plantations the fungus severely attacks the most vigorous (dominant) as well as the weakest trees, but after analyzing the local conditions it was found that these strong-growing trees were not so vigorous as they should have

been and that the site in general was more or less unfavorable for Asiatic chestnuts. Near Mount Cuba, Delaware, a forest planting of a single strain of Chinese chestnut was made in the spring of 1926 on well-drained Chester loam. This planting is surrounded on 3 sides by native forest trees and the land planted to chestnut had formerly been in cultivation. The trees at the lower edge of the plantation have made excellent growth, averaging almost 3 feet increase in height a year. They are very straight and are surrounded by natural reproduction of tulip poplar, black birch, hickory, butternut, and black walnut. Trees in other parts of the plantation where the ground is covered with sod are shorter and more branched. Natural forest reproduction of hardwoods in this part of the site has failed to develop, although some seedlings have appeared and lived for a season or two before dying. A detailed comparison was made between an equal number of vigorous (dominant) chestnuts growing in association with natural reproduction of hardwoods and less vigorous (suppressed) chestnut trees growing in the open field. *Cryptodiaporthe castanea* was present on trees of both classes, but on the dominant ones it was confined almost entirely to the shaded lateral branches and many cankers had been healed over by callous formation. No appreciable amount of girdling was found on these trees and the organism was weakly parasitic. Numerous cankers were observed on the lateral branches and a few on the trunks of the suppressed trees. Some of these cankers had also been healed over by callus, but many were still active and some had girdled. It is believed that the disease does not seriously injure the trees except when trunks or major branches are girdled.

At Willets, N. C., a forest planting of the same strain of Chinese chestnut was made in the spring of 1926 on a north-facing cove site of well-drained Porter loam among small native hardwoods that had been thinned. The dominant chestnut trees in this plantation averaged from 2½ to more than 3 feet in height growth each year, while those suppressed by competition with other trees were stunted in comparison. A detailed examination of an equal number of dominant and suppressed chestnut trees in this plantation gave results similar to those obtained at Mount Cuba.

Cankers have been found on branches and trunks several inches in diameter, but no girdling has been observed on branches more than 1½ inches in diameter.

The imperfect stage of *Botryosphaeria ribis* has been collected on dead American chestnut twigs at several localities. It is also known to occur on various hosts over a considerable range. Stevens (17) stated that this currant cane-blight fungus is apparently fairly common on a variety of hosts, even in tropical and subtropical regions more than a thousand miles from the region where the currant is grown commercially, and suggested that the fungus was originally confined to native hosts of southern range. Smith (16) found that the host range of this fungus in nature includes at least 34 genera and 20 families of plants.

Very little is known about the potential damage that may be caused by the *Botryosphaeria* or *Diplodia* on Asiatic chestnuts, as they have been found on these hosts so infrequently that detailed studies under natural growing conditions have not been possible. Greenhouse inoculations indicate that they may be serious parasites, but conclusions based on artificial inoculations may be erroneous.

CONTROL

Investigations have shown that the disease induced by *Cryptodiaporthe castanea* is most prevalent and severe on weakened trees; hence the most important measure of control is to maintain high vigor of individual trees and of stands. Diller (6) recommended that forest plantings of Asiatic chestnuts be made on deep, well-drained, fertile soil suitable also for the establishment of volunteer hardwood seedlings such as tulip poplar, ash, hickory, and oak. It is believed that if the site on which chestnuts are to be planted is carefully selected so as to provide optimum growing conditions and the trees are carefully planted, the disease will not assume serious proportions. In orchard plantings the same care should be exercised in the selection of sites, but the possibility of fertilizing and cultivating the trees to maintain vigor may be taken into consideration.

Artificial inoculations and field examinations demonstrate that all three fungi may infect their hosts through wounds. Therefore, wounds should be avoided if possible, or if induced by pruning or in other ways, should be protected by a wound dressing such as asphaltum paint.

Since these fungi overwinter on cankers and dead twigs, all infected stems should be removed and burned during the early summer when the disease is easily recognizable. Stems should be pruned several inches below the infected area to make sure that none of the disease remains. Badly infected trees can be cut off near the ground and allowed to sprout from the roots, but should be uprooted if the infection extends to the base of the tree. These control measures are particularly emphasized for trees growing in nurseries and may possibly be supplemented by spraying.

Spraying nursery stock with a fungicide is now being tested, but as yet the results are too uncertain to permit recommendations. Means of spread of the fungi are not known.

SUMMARY

Asiatic chestnuts planted on poor sites have been found to be severely affected by canker and dieback diseases, while those planted on sites permitting optimum growth have not yet been found seriously affected. Several genera of fungi are represented by the fungi found associated with the dead twigs. *Cryptodiaporthe castanea, Botryosphaeria ribis chromogena,* and *Diplodia* sp. have been shown through inoculation experiments to be capable of invading living tissue and producing cankers and diebacks of Asiatic chestnut twigs.

Cryptodiaporthe castanea, the fungus most commonly found associated with the cankers of Asiatic chestnuts in the United States, has been found in 18 states and the District of Columbia. It has been present in 30- to 40-year-old Asiatic chestnut orchards for some time. It is not known whether this fungus is a native of this country. It was originally described on *Castanea sativa* in Europe and, if introduced, it has apparently been here for some time. It is sometimes damaging in nurseries and may cause the death of planted trees. It more frequently kills individual branches, thus decreasing growth and deforming the trees. The other two fungi have been collected only a few times and as yet are not known to be of great importance.

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ARTIFICIAL PRODUCTION OF "BLACKHEART" IN POTATO TUBERS

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It was first shown by Bartholomew (1) that blackheart could be artificially produced by subjecting the tubers to a temperature between 38° to 48° C. for 14–48 hours. Stewart and Mix (2) accidentally discovered that "by excluding the air from potatoes, blackheart may be produced at temperatures much lower than those employed by Bartholomew." They found that the length of time required to produce the disease was increased with an exposure of tubers to lower temperature, but it developed, even at 40° F. after 23–40 days, when the volume of air in the container was equal to the volume of the potatoes. It has been claimed that the injury resulting from poor aeration is due to a lack of oxygen and not to the accumulation of carbon dioxide given off during respiration. In this connection it is striking to note that Davis (3) was able to produce blackheart symptoms in potatoes, even when stored in CO_2-free air with an abundance of oxygen.

In connection with our work on the diastatic and oxidase activities of blackheart-diseased tubers (4), in the summer of 1935, we were able to produce typical blackheart symptoms by enclosing the tubers in hermetically-sealed jars at 30–56° C. for 6 days. But it was observed occasionally that the percentage of diseased tubers in potatoes sealed in 4-liter containers and heated at a given temperature for a fixed number of hours was extremely variable. This led us to believe that the condition of the tubers prior to heating with a view to produce blackheart was probably an important factor and that the variability in the results obtained was referable, in all probability, to our using a mixture of potatoes of various developmental stages. Moreover, in our work on the storage of potato tubers (5) it has been shown that the developmental stage of the tuber is a factor of considerable physiological significance. An experiment, therefore, was designed to test the relation between the developmental stage of the tubers and the percentage of blackheart induced at various temperatures.

During the growing season and the storage period of 1935–36, 6 samples were taken. Experiments were first performed on Dec. 1 and thereafter on Dec. 28, Jan. 21, March 10, May 20, and June 15. The work on the respiratory drifts and chemical composition (6, 7) showed that the tubers sampled on the above-mentioned dates belonged to the following more or less well-defined stages: adolescence, maturity and ripening (during the growing season) and early dormancy, middle dormancy, and late dormancy (during storage). Experiments were performed at 40.3°, 48.1°, 52.7° and 57.1° C. and the duration of heating varied from 48 to 105 hours. In all cases large-size tubers (var. Katua) were packed in sealed 4-liter containers, leaving a minimum of gas space. Relevant data are recorded in table 1.

TABLE 1.—*Relation between the developmental stage and the percentage production of blackheart at various temperatures*

Temp. °C.	Duration of heating (hours)	Developmental stage					
		Adolescence	Maturity	Ripening	Early-dormancy	Middle-dormancy	Late-dormancy
40.3 ± 0.3	48	No disease	No disease	No disease	No disease	20.2	22.7
"	70	"	"	"	"	41.7	41.2
"	90	"	"	"	"	46.2	37.2
48.1 ± 0.4	48	"	"	"	8.2	41.0	10.5
"	70	"	"	"	10.7	48.7	No disease
"	90	"	"	0.9	25.2	48.2	15.9
"	105	"	"	0.7	27.7	47.3	11.7
52.7 ± 0.5	48	"	"	1.2	27.0	47.2	No disease
"	70	"	0.1	1.7	30.1	50.2	50.2
"	90	"	0.4	2.0	35.2	54.7	10.7
"	105	"	No disease	2.1	35.1	56.2	15.7
57.1 ± 0.5	48	"	2.0	2.7	No disease	37.3	No disease
"	70	"	2.1	2.1	40.2	40.7	40.7
"	90	"	2.8	2.9	35.8	41.2	40.2
"	105	"	3.7	4.8	40.3	55.7	39.9

It is evident from the data that during adolescence, *i.e.*, when the tubers are very small and actively growing, even heating them at 57.1° C. for 105 hours could produce no visible signs of disease. On the contrary, during maturity, when the potatoes have attained more or less the maximum size, 0.1 per cent of the tubers were found to have contracted the disease when heated at 52.7° C. for 70 hours. During ripening, *i.e.*, when the potatoes had been in the soil for about 10 days after the stoppage of growth, 0.9 per cent of the tubers were found to de diseased in the lot heated at 48.1° C. for 90 hours. During early dormancy first signs of blackheart were observed in tubers maintained at 48.1° C. for 48 hours, whereas during middle dormancy 20.2 per cent of tubers were found to be diseased even when heated at 40.3° C. for 48 hours. Although no experiments were performed to test the assumption, it is very likely that the minimum temperature and duration for producing disease in potatoes in the middle-dormancy stage are much lower than 40.3° C. and 48 hours. The data obtained during the late-dormancy stage of the tubers show no general trend and are rather contradictory among themselves. For example, heating of potatoes at 52.7° C. for 70 hours produced 50.2 per cent diseased tubers, whereas the lot heated at the same temperature for 105 hours produced only 15.7 per cent diseased potatoes. This anomaly appears to be attributable to the fact that, during late dormancy, processes leading to sprouting had already commenced, and, although no sprouting tubers were included in the experiments, it is easy to imagine that the physiological and chemical changes inside the tubers preparatory to sprouting begin fairly in advance of the external appearance of sprouts, and were, on this account, probably included in the lots for experimentation undetected.

A consideration of the degree of susceptibility of adolescent and mature tubers to blackheart suggested the possibility of the size of the tuber being a factor in the incidence of blackheart. On May 20, 1936, some experiments

TABLE 2.—*Relation between the weight of potato tubers and their susceptibility to blackheart*

No. of tubers examined	Mean fresh weight	Percentage of diseased tubers
	Variety Katua	
53	84.2	71.3
58	79.2	62.9
92	65.7	39.2
97	59.2	37.6
102	41.1	32.1
140	35.1	30.9
210	29.8	30.7
	Variety Farrukhabad	
31	66.2	30.2
60	45.1	22.3
95	33.2	15.2
141	30.1	14.7
219	2.6	5.0
239	1.4	4.7

were performed on potatoes that, according to later respiration determinations and chemical analysis, were found to belong to the middle-dormancy stage. Two varieties of potatoes, Katua and Farrukhabad, were divided into a number of groups on the basis of their fresh weights. The potatoes belonging to each group were separately heated at 57.1° C. for 105 hours and the percentage of tubers showing signs of disease calculated. Relevant data are recorded in table 2. There is an indication that the bigger potatoes are more susceptible to blackheart than the smaller ones.

To sum up. Of potatoes of the various developmental stages those in the middle dormancy stage are most susceptible to blackheart. There is an indication that the smaller tubers are less likely to contract disease than the bigger ones.

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AGE OF SUSCEPTIBILITY OF RIBES PETIOLARE LEAVES TO INFECTION BY AECIOSPORES AND UREDIOSPORES OF CRONARTIUM RIBICOLA

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INTRODUCTION

Studies on the relative susceptibility of various native species of *Ribes* to infection by white pine blister rust (*Cronartium ribicola* Fischer) were among the first undertaken when the disease was discovered in eastern North America. In these early studies it soon became apparent that the age of the leaf at the time of exposure to infection is an important factor in its susceptibility.

Spaulding (8, p. 45) summarized his own observations and those of other eastern investigators up to 1919 and concluded that the age and relative maturity of the leaf are important factors in its susceptibility. The most favorable stage of growth, although there is considerable variation between species, seemed to be reached at about the time when the leaf attains full size, but has not become hardened and leathery as it does later.

In greenhouse inoculations of Pacific northwestern *Ribes* with urediospores of *Cronartium ribicola* and the native piñon blister rust (*C. occidentale* Hedgc., Beth., and Hunt), Hahn (2, p. 679) found that normal uredia were developed readily on fully developed leaves of *R. petiolare* Dougl. The uredia produced on immature leaves just below the growing shoot tips, however, were undersized and associated with necrotic flecks. This resistant type of infection was encountered also on the immature leaves of *R. viscosissimum* Pursh., *R. howellii* Greene, *R. laxiflorum* Pursh., *R. lacustre* (Pers.) Poir, and *R sanguineum* Pursh. A somewhat different reaction was encountered when *R. triste* Pall. was inoculated. In preliminary tests Hahn (2, p. 679) found that leaves of this species showed a marked tendency to become infected for only a very limited period. Very young leaves produced necrotic flecks only; fully expanded leaves were susceptible but these leaves soon hardened and became apparently immune.

Inoculation results similar to those previously reported for *Ribes triste* were obtained by Hahn (3, p. 107) in a subsequent greenhouse test of several varieties of red and white garden currants with urediospores of the white pine and piñon blister rusts. He found that immature leaves did not become infected, nor did leaves that had commenced to harden.

Leaves of the susceptible *Ribes* used as "checks" in experiments with the blister-rust-immune Viking currant from Norway showed the same relationship between tissue development and rust infection (4, p. 9) as that encountered with leaves of *Ribes petiolare* and other Pacific northwestern *Ribes* previously studied (2, p. 679).

Lachmund (6) reported the results of studies conducted in the West in which *Ribes petiolare, R. inerme* Rydb., *R. viscosissimum,* and *R. lacustre,*

the principal *Ribes* associates of western white pine, *Pinus monticola* Dougl., were artificially inoculated in the field with aeciospores of *Cronartium ribicola.* These results indicated that the leaves were susceptible from the time they first emerge from the buds and that the period of highest susceptibility lay, in general, between the ages of 2 and 16 days (p. 113). As the leaves became older, their susceptibility to blister rust infection declined.

The results of the western study did not substantiate, in all details, those secured from the various studies conducted in the East. This lack of uniformity may be explained in part by the differences in methods employed. The eastern studies were conducted largely in the greenhouse with artificially propagated plants, and the inoculations were generally made with urediospores. The western study, on the contrary, was conducted on native plants growing out-of-doors, and aecial inoculum was used.

Lachmund (6, pp. 111–113) has shown that "the most favorable period for spread of the disease by aeciospores occurs when the period of maximum spore dispersal from a given center synchronizes with that in which the largest proportion of the leaves lying within the long-distance range of the spore showers are in their most susceptible stages." It is apparent, then, that the relative susceptibility of the *Ribes* leaves at time of inoculation is an important factor in their infection and, consequently, also, in the spread of the rust. In view of the somewhat contradictory results secured from eastern and western studies it was considered desirable to obtain additional information on the relationship between age of leaf and susceptibility. For this purpose the study herein reported was conducted at Moscow, Idaho, and methods more comparable to those of previous eastern studies were employed.

ORIGIN AND CARE OF TEST PLANTS

Ribes petiolare was used as the test plant in this study, since it is one of the most important *Ribes* species within the commercial range of western white pine (9), ranking fourth in abundance and first as introducer of the rust into a locality. The high susceptibility of this species has been demonstrated (2, 7) and data on the age of susceptibility of its leaves are available from both eastern (2) and western (6) studies.

The individual plants used in this study were collected near Clarkia, Idaho, in November, 1933. All were natural seedlings, 1 year old and from 3 to 5 inches in height. Fifty such plants were collected, potted in forest soil, and held over winter in the greenhouse[1] at Moscow, Idaho. At the beginning of actual experimental work the plants were stripped of their leaves and all lateral buds were removed. Of the 50 plants available, 30 individuals whose terminal buds burst at approximately the same time (February 10–11, 1934) were selected for the study.

RIBES LEAF DATA

The first series of leaves was tagged on February 15, 1934. These leaves had emerged from their buds within 5 days of each other and were mostly

[1] Greenhouse and laboratory space was provided through the courtesy of the School of Forestry, University of Idaho.

from 1 to 4 days of age, all having appeared between February 10 and 15. Every fourth day thereafter all new leaves were tagged with minute paper tags so as to distinguish between leaves of different 4-day age classes. Seventy-two days after the first leaves emerged from their buds the last series of leaves was tagged. A total of 18 consecutive 4-day age classes of leaves had thus been tagged; the leaves in the 69- to 72-day age class were those that first emerged from their buds. At least 8 of the age classes were represented on every plant; many individuals had nearly all age classes represented; and in every case, there was a difference of at least 53 days between the age of the youngest and oldest leaf on any plant.

On April 24 the last series of leaves was tagged and a leaf inventory was taken. The 30 plants were then divided into two groups, each of which had approximately the same number of leaves of all age classes represented. Between late evening, April 24, and early morning, April 25, a print was taken of each leaf by exposing a sheet of sensitized paper while closely appressed to the flattened leaf. These prints, when later planimetered, gave the area of leaf surface of different age classes at the time the last series of leaves was tagged.

INOCULATION OF RIBES

The aeciospores, with which one-half of the plants were inoculated, were collected on April 18, 1934, from blister-rust cankers on *Pinus monticola* in a natural infection area along Crystal Creek near Fernwood, Idaho. The urediospores, with which the remaining plants were inoculated, were collected from stock cultures maintained on *Ribes petiolare* in the greenhouse.

On the night of April 26, aqueous suspensions having a density of 100,000 spores per cubic centimeter were made up for each of the two spore types. Fifteen of the plants were then inoculated with the aeciospore suspension. Each leaf was inoculated individually, while shielded from the remaining leaves, by giving the lower surface 2 blasts from a De Vilbiss atomizer held at a constant distance from the leaf. This distance was sufficiently great to cause the spore suspension to reach the leaf in the form of a dense vapor rather than as a definite stream, thus insuring uniformity of application per unit area irrespective of size of leaf. All leaves on the other 15 plants were inoculated in a similar fashion, but with the urediospore suspension. The application was checked by spraying the spore suspensions onto glass slides in the same manner in which the leaves had been sprayed. An examination of these slides showed that a comparable number of each type of spore had been applied per unit of leaf area. A bell jar was placed over each individual plant immediately after inoculation and left there for 36 hours to provide more favorable infection conditions. After removal of the bell jars the inoculated plants remained in the greenhouse until the study was completed.

RESULTS

On May 7 and 8 the leaves were stripped from the bushes and dried in a plant press. The length of time since inoculation (11 to 12 days) was con-

sidered sufficient to permit the vast majority, if not all, leaf flecks to make their appearance, Spaulding (8, p. 40) having shown from greenhouse tests that, in 63 per cent of the cases, 12 days was ample time for infections to develop into mature uredia and that all infections developed mature uredia within 17 days. Observation showed that none of the infections on leaves of the test plants had developed far enough to produce urediospores that might have intensified the rust.

After the leaves were dried the flecks indicative of infection by *Cronartium ribicola* (1, p. 634; 5, p. 874; 8, pp. 53–54, Pl. VI, Fig. 2) were carefully counted for each of the 18 leaf age classes represented. For purposes of comparison the number of leaf flecks in each age class was converted to a flecks-per-10-square-centimeter basis. Fertile uredia may not have developed eventually from every leaf fleck. From previous infection studies (6, 7), however, involving thousands of infected *Ribes petiolare* leaves, it has been observed that leaf flecks develop fertile uredia with equal facility when originating from either aecial or uredial infections. In determining the relative susceptibility of different aged *R. petiolare* leaves the relative number of leaf flecks developed is, therefore, a reliable criterion.

All inoculated plants and inoculated leaves of all ages became infected, although every leaf inoculated did not become infected. Table 1 shows the final results of this study for both the aeciospore and the urediospore inoculations. With both spore types the highest susceptibility (as judged both by percentage of leaves infected and by number of leaf flecks per unit area) was found on leaves in the younger age classes. A decrease in susceptibility was found to accompany increased leaf age irrespective of spore type used in making the inoculation. Results of the aeciospore test showed leaves up to and including those in the 9 to 12-day age class to be decidedly more susceptible than older leaves. With urediospores, however, this high susceptibility definitely extended to leaves in the 13- to 16-day age class and, to a lesser extent, leaves even as old as those in the 21- to 24-day age class exhibited fairly high susceptibility.

It is interesting to compare these ages of highest susceptibility with leaf development. By combining all leaves of a given age class and computing their average size, it was found that the *Ribes petiolare* leaves apparently required about 3 weeks to reach size-maturity after emerging from the buds. It must be realized, however, that there is great individual variation in leaf size at maturity and that this individual variation accounts for the differences between sizes of leaves in the aeciospores and urediospore tests, as are shown by age classes in table 1. The data do indicate, nevertheless, that the *Ribes* leaves were in their most susceptible state during the first few days after emergence and that they remained highly susceptible until about the time of size-maturity, after which their susceptibility declined. This conclusion is in close agreement with that reached by Lachmund from field studies previously reported (6, p. 113).

Extremely young leaves became infected in both the aeciospore and the urediospore tests. Unfortunately, there was at least a 4-day range in each

TABLE 1.—*Age of susceptibility of Ribes petiolare leaves to infection by Cronartium ribicola as determined by greenhouse inoculations with aeciospores and urediospores*

Leaf[a] age class	Aeciospore inoculation							Urediospore inoculation						
	Total plants	Number of leaves		Leaf area[a]		Rust flecks	Flecks per 10 sq. cm.	Total plants	Number of leaves		Leaf area[a]		Rust flecks	Flecks per 10 sq. cm.
		Total	Infected	Total	Average				Total	Infected	Total	Average		
(Days)	*No.*			*Sq. cm.*	*Sq. cm.*	*No.*	*No.*	*No.*			*Sq. cm.*	*Sq. cm.*	*No.*	*No.*
1– 4	11	19	17	39	2	622	158	13	18	15	36	2	457	127
5– 8	13	16	16	164	10	2244	137	11	13	13	103	8	1844	179
9–12	13	17	17	302	18	3037	101	9	10	10	168	17	2522	150
13–16	11	13	13	356	27	1656	47	12	14	14	320	23	3857	120
17–20	11	12	12	349	29	1297	37	11	11	11	352	32	3379	96
21–24	9	11	11	281	26	917	33	9	11	11	380	35	3713	98
25–28	9	9	9	243	27	449	18	5	5	5	151	30	798	53
29–32	7	7	6	198	28	441	22	10	10	10	301	30	1386	46
33–36	8	9	8	222	25	623	28	10	10	9	300	30	868	29
37–40	7	7	7	196	28	329	17	9	10	9	325	33	892	27
41–44	9	9	9	213	24	511	24	12	12	10	294	25	936	32
45–48	13	14	13	326	23	489	15	9	12	9	296	25	785	27
49–52	11	15	12	410	27	567	14	11	13	11	296	23	280	9
53–56	10	14	10	229	16	294	13	8	16	14	371	23	512	14
57–60	10	13	9	262	20	214	8	10	16	8	247	15	284	12
61–64	9	11	7	331	30	326	10	6	8	3	210	26	26	1
65–68	12	16	10	575	36	583	10	13	18	10	458	25	266	6
69–72	11	14	8	570	41	272	5	12	18	14	622	35	254	4

[a] Age and area at time of making leaf prints (April 24–25). Since the inoculations were not made until April 26, the leaves, especially those in the younger age classes, were slightly larger than is shown. Similarly, the 1- to 4-day age class includes leaves actually 2 to 6 days of age, and the leaves in all other age classes are 2 days older than the designated extremes.

age class, making it impossible to state definitely that the leaves were susceptible immediately upon emergence from the buds. After exhibiting comparatively high susceptibility to *Cronartium ribicola* infection while very young, the leaves of *Ribes petiolare* became less and less susceptible (or more and more resistant) as they became older. This was true irrespective of type of spore used as inoculum. Although the trend was somewhat erratic because of the small basis in certain age classes, it was definite and was maintained until the leaves were at least 71 to 74 days old (69- to 72-day age class). Under greenhouse conditions, even the oldest leaves tested did not present the hardened, leathery appearance overmature leaves exhibit as they develop under field conditions. Since these leaves were relatively low in susceptibility, it would seem that something in addition to leaf succulence is responsible for the differences in susceptibility found between leaves of various ages.

Table 1 shows that both aeciospores and urediospores of *Cronartium ribicola* are capable of causing infection on *Ribes petiolare* leaves of all ages, from those but 2 to 6 days old to those 71 to 74 days old. No attempt should be made, however, to draw conclusions from this table as to the comparative infecting ability of these 2 spore types. The factor of spore viability was not sufficiently well controlled to permit such a comparison to be drawn.

SUMMARY

The leaves produced on 30 *Ribes petiolare* seedlings, maintained in the greenhouse at Moscow, Idaho, were tagged at 4-day intervals until leaves in all age classes up to those in the 69- to 72-day age class, inclusive, were represented. The plants were then divided into 2 equal groups and the area of each leaf determined. On April 26, 1934, 2 days after making the area determinations, one group of these plants was inoculated with an aqueous suspension of *Cronartium ribicola* aeciospores and the other group with a similar suspension of urediospores of the same heteroecious rust. After an incubation period of 11 to 12 days the leaves were stripped from the plants and the flecks indicative of infection were counted.

All plants and leaves of all ages became infected. The relative susceptibility of leaves of different ages was determined by comparing the number of flecks per unit area at the approximate time of inoculation. The younger leaves were found to be the most susceptible to infection from both spore types. Leaf susceptibility remained high until about the time of size-maturity (approximately 3 weeks after emerging from the buds). After reaching size-maturity an increase in leaf age was accompanied by a decrease in susceptibility.

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SOME ASPECTS OF THE CONTROL OF SPIKE DISEASE IN SANDALWOOD

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INTRODUCTION

Spike disease in sandal represents a diseased condition of the leaves and twigs. It manifests itself in an abnormal vegetative activity of the plant and is diagnosed by a characteristic reduction in leaf size, and by an extremely striking disposition of the internodes at regular but short intervals. With this enhanced vegetative growth as evinced by excessive branching of the diseased parts, the reproductive phase is entirely inhibited in them. Even the few fruits that may be formed are incapable of germination. Moreover, the diseased plants eventually die. In view of this interesting feature about this malady, which appears to threaten the existence of this economically important species—a monopoly of South India—some Governments have spent time and money in discovering a proper solution for it. An attempt is made here to review our present knowledge in controlling this pest.

The necessity for controlling the disease arises from the fact that it is highly infectious in character, as shown by Coleman (7) and by Sreenivasaya and Naidu (14). In other words, the disease could be reproduced at will artificially. In the absence of bacteria, etc., and in the light of evidence presented by Narasimhan (11), it is considered to be virus in nature. Though its origin remains obscure, it has made considerable advance into many of the sandal forests within the last 40 years. As sandal is one of the important revenue sources for Mysore, Coorg, and Madras, the advent of spike disease has been a curse. The spread has been in a southeasterly direction, causing much

havoc to the several localities. The rate of attack has varied enormously. As a rule it spreads slowly and steadily, but in some years, the attack has assumed highly virulent and epidemic proportions. There are areas where the infection exists in such virulent form that it had proved a hopeless problem for the forest departments. In a similar way, infectivity varies in individual plants. While it is normal to see a plant with 1 or 2 shoots primarily affected, several instances have been observed where the crown is diseased all over, simultaneously. Thus, there is no knowing when an area is ready to be infected and, if infected, whether the attack will be a mild one or will assume epidemic proportions. All these observations demand a prompt action for controlling spike disease, if sandal is still to be the monopoly of the Deccan plateau.

ELIMINATION OF FACTORS CONDUCIVE TO THE SPREAD OF INFECTION

Although spike disease is ultimately fatal, the affected tree continues to put forth its characteristically reduced leaves for a maximum period of 3 years, after which it dies. Therefore, even one infected plant is a veritable source of infection to its healthy neighbor. The mechanism of this transference of disease is varied, apart from the fact that such centers of infection are varied also. Unless, therefore, the radiating points of infection are rooted out, the problem of controlling spike disease is not solved. In what manner can this be done at a cost that will admit of ready adoption, in addition to its being efficient also, will be treated here.

Through the Removal of the Diseased Plant Itself. The suggestions of Butler (5) to create a belt of 100 feet around an infected patch was found to check the spread of disease only for a time. This is feasible primarily in incipient stages of attack and also when small patches are to be treated. On the other hand, mechanical removal and complete destruction of spiked plants has involved a large expenditure without corresponding quickness in effect. But where this procedure has been adopted with promptitude, effective check on the rate of infectivity has been recorded. This is the real clue to the problem on hand. The practice of awarding one anna for every spiked plant pulled out, once cost the Madras Government an enormous sum of money. The difficulty here was one of identifying whether a plant actually was affected or not; obviously, even healthy ones were removed. All the same, this was the only recognized method. If, therefore, the diseased plant can be effectively eliminated, the problem is somewhat easier to deal with.

Where this plant removal has not effectively been carried out, troubles have arisen. For instance, sandal is known to produce coppice shoots, if the trunk is lopped. These shoots are more numerous but concentrated, with reference to the active principle, when the spiked plant is cut at its bole. Another feature of sandal is its readiness to produce root suckers, which have their origin in the cut portions of roots. Such plants are bound to arise; since the removal of roots by mechanical means is never complete. It is known also that roots of diseased trees carry the infective principle in

them. Spiked plants also produce root suckers which are diseased and hence contribute to further spread of infection. Thus, there is provided a ready means of establishing the disease. The question of root suckers in sandal is something extraordinary. Thus, in Coorg, the writer came across 2 sandal plants 1 on either side of a road, differing from each other in height. On examination it was found that the smaller plant was a root sucker from the bigger one. Again, in the course of some experiments on artificial transmission of spike in another locality, certain uninoculated plants revealed symptoms of spike, but were later on discovered to be root suckers from the treated ones. These raise problems of great significance as to whether, during the incubation period, the roots are primarily infective, or whether the infective principle is carried to the root ends. In any event, the elimination of such plants is the most immediate problem. It is evident from this, that an incomplete removal of the diseased plant does not prevent the spread of spike.

It was recorded fairly early that sandal was a root parasite. But we owe it to the late Dr. Barber (2) who elaborated it in his extensive researches on sandal in relation to its nutritive requirements. He also showed that sandal grew best in the presence of host plants for a period of at least 5 years (3), a fact that has since been corroborated by Sreenivasa Rau (13). But Sir Dietrich Brandis' (4) question whether sandal does not derive a part, though little, of its nutrition from the soil also, has not adequately been answered. It is probable that sandal roots derive some nutrition through the root caps, in the absence of root hairs. In view of the capacity of sandal to form haustoria on its own roots, it is of significance to find that healthy plants also can be infected possibly by parasitizing the roots of a spiked plant. Such a possibility was indicated by Coleman (8), although our own tests in the Indian Institute of Science, Bangalore, under artificial conditions, have thus far been unsuccessful in this direction. This is one more reason why the diseased plant should be entirely eliminated as early as possible.

It has often been said that spike is a purely physiological manifestation arising out of unfavorable environmental conditions. By a comparative study of infected and healthy areas, Varadaraja Iyengar and Rangaswami (20) showed that the death rate of sandal in the former was abnormally high. The further fact that localities far distant from one another also are affected indicates the infectious nature of the disease. Great significance also is attached to the beneficial results noticed in areas from which spiked plants have been removed. A series of investigations on the control of spiked trees by the above authors (20) has led to the conclusion that arsenicals are the best plant poisons for the purpose. Others also may act equally well, but their high cost, for example, that of sodium chlorate for application in greater concentrations, is not commensurate with the results secured. The success of the new procedure has been well established. Thus, several localities, where spike has been reported in incipient stages, have been saved from the invasion of this pest. In other words, but for this simple technique and

process developed by them, many of the sandal areas would have been doomed. Sodium arsenite is reasonably cheap and is now available on the market in a highly improved condition evolved by the writer.

The problem now resolves itself into a control of areas both mildly and virulently affected. In the former case, a prompt action based on the above technique has proved successful. Where healthy trees are few compared with diseased ones, or where the rate of spread of spike is epidemic in character, the problem is more complicated. Although it may be argued that the disease itself may exterminate the species within a few years, experience does not corroborate this view. There are several blocks where sandal is prolific and the disease also is fairly active. There is no record of any area having been entirely wiped out in this manner. In such cases bold action is essential. It is true that the cost of treatment in such localities is high. But the advantage lies in eradicating the infection center at a shorter period than can be imagined. It is, therefore, necessary that a concerted attack in controlling the spread of disease should be made, if the monopoly of sandal is to be retained. The cost involved in this is really worth while. A special staff may have to be temporarily appointed for this purpose. It may be remarked here that on an average 150 trees can be treated with one gallon of the solution. Hence an enumeration must first be made to compute the necessary amount of money required. It is urged that the Governments concerned will take up this question at once.

Without going into greater details, it may be remarked that our tests with the poison on diseased plants have proved effective and innocuous. The effect of the poison is visible within a week to a fortnight, depending on the season of application. Where the first dose has failed—this may be due to want of care in treating—a fresh application should be made. In some cases of virulent infection, a different mode of treatment will be better. The coppiced stumps respond well to the poison when applied on the cut surface. In any event, it was found through experiments that both the heartwood and the oil obtained therefrom contained essentially no arsenic (17). In other words, the therapeutic value of these commodities does not appear to have been impaired by this method to any extent whatever. It is also on record that by this treatment, root suckers of diseased plants have been killed outright. It is claimed, therefore, that the above process is the best for controlling the disease in the quickest possible time.

(b) *Through the Removal of Possible Alternate Hosts of Sandal Spike.* It is well known that there are several species occurring in the forest side by side with sandal, and many of these are found to exhibit spike-like symptoms, the nature and cause of which are yet obscure. *Zizyphus oenoplia, Dodonea viscosa, Vinca rosea* and a few others are the most important of these, although the total number of such manifestations is nearly 40. Many more have to be included in this list. It is of significance, however, to find that spike in *V. rosea* is almost identical with that in sandal as shown by Narasimhan (11) and by Varadaraja Iyengar (18). Whether all these were

attacked simultaneously with sandal, is too much to say. But one thing is certain; that these diseased plants are to be seen in localities where the sandal is not known to grow. Such definite instances have been recorded by Barber and also confirmed by the writer. More cannot be said on the identity of these. Even though the symptoms do not closely agree in all respects, different species may react in varying manner to the active principle. The possible relation of these acting as alternate hosts to sandal spike cannot, therefore, be ignored. Our own evidence on it from artificial-transmission studies is weak. That insects may be better fitted to effect such transferences is well recognized. In order therefore to prevent the spread of infection in sandal, such diseased plants must be eliminated. The author has tried sodium chlorate for the purpose. But in those cases where the plants are big, like those of *Z. oenoplia*, preparations containing sodium arsenite have been successfully applied, as with spiked sandal. It is, however, recognized that more attention should be paid to this aspect of the problem. Besides these, the possibility of other virus infections of plants serving as alternate hosts to sandal spike, has to be eliminated, but in the writer's opinion, it is only of secondary importance.

(c) *Through Factors Relating to the Carrier of Infection.* A preliminary study on this point was made by Hearsey (10) and reported in 1917, in which he could not incriminate any particular species with the transmission of spike. In a similar way, Chatterjee (6) failed in his attempt, although he has contributed a great deal on the insect relations of sandal. But Dover announced (1) that one of the sap-sucking type of insects (*Moonia albimaculata*) was responsible for transferring the disease to a healthy plant in pots almost at the close of the spike investigation scheme. Much controversy arose over his discovery. His evidence was based on the presence of X-bodies in such leaves as per technique of Narasimhan and the nitrogen content of the same specimens. In dealing with these tests one may point out that nitrogen values are never reliable for such purposes, and that he should have secured data for calcium and the calcium/nitrogen ratio, the surest index. The data for biometric measurements, referred to in a later publication (9), have been critically examined by the writer elsewhere (19). Suffice it to point out here that the writer's own measurements on them corresponded with those for healthy specimens. A similar examination served to eliminate Chatterjee's claims of a beetle as a possible carrier (19). It is indeed doubtful whether Sreenivasaya's technique of lopping these plants is a reliable one, and will be referred to later.

The real transmission of spike through insects was discovered by Rangaswami and Sreenivasaya (12) who showed that under cage conditions, healthy plants in pots exhibited symptoms of disease when a mixed fauna gathered from the forests was dumped into it. They claimed that the night collections were those responsible for this transference. Further experiments with individual groups and species have not, so far, been successful. The mass-feeding series was repeated with success (15).

As long as our knowledge is obscure on the insect to be incriminated with the transmission of disease, attempts to control the carrier of infection will be futile—nay, even unwise. It is known that treatments differ depending upon the nature of the fauna involved. Two methods of control are recognized, biological and biochemical. In the former case, the necessary parasite is introduced to inhibit the development of the insect vector. In the latter, both diseased and susceptible plants are sprayed with chemicals, the nature of which differs with the type of insect. In either case, the writer agrees with Dr. Butler (private communication) that control of spike disease is a very costly process.

IDENTIFICATION OF DISEASE

Whatever treatment may be recommended for spike, there is one aspect that has not been adequately recognized. It relates to the identification of the potential source of infection. In other words, the symptoms generally described and understood are not sufficiently indicative of the distinction between a healthy and spiked plant. While it is normally easy to identify affected ones, many instances have been brought to the writer's notice that have led to some confusion, even among experienced workers This is complicated by the fact that growth in sandal is conditioned to a great degree by its parasitic habit and hence, by the nature of the host plant associated with it. In several areas, healthy trees have been noticed with foliage apparently resembling spiked ones. Some of these have been referred to elsewhere by the writer (19). In order to eliminate the difficulties normally encountered, a simple technique of a rough and ready nature was evolved by the author. This has stood the test quite well in the field, being based on leaf and internodal measurements. A more accurate and definite index was discovered by the author on the physiological data secured from different areas for a large number of specimens. Thus, the ratio Ca/N is the safest for the purpose (19); in fact, failure to obtain such data for those cases wherein transmission of spike through insects has been claimed has resulted in some confusion.

A point of equal importance is that of disease masking. It is claimed by Sreenivasaya that certain apparently healthy plants develop symptoms of spike when they are lopped, *i.e.*, deprived of their crown. The basis for this arises out of some experiment in which artificially infected plants were found to manifest the disease on being lopped. No evidence is available as to the particular stage following inoculation when this is reproducible. Evidence also is lacking on the possible infective nature of such specimens by grafting and other tests. There appears to be some confusion on this point. One ought to distinguish between incubation period and disease-masking stage. In fact, scores of experiments carried out at Noganoor revealed the astounding feature that certain plants did not exhibit spike symptoms even after 380 days. It is therefore naturally felt that lopping is not a wise technique. Moreover in some of the earlier experiments on controlling spread of spike,

only those trees that manifested the disease were removed with the result that the area was later completely free from infection for several years. This aspect of the problem is quite complex, requiring elucidation of several points based on extensive experimentation.

FACTORS RELATING TO SUSCEPTIBILITY AND RESISTANCE TO SPIKE INFECTION

It was remarked earlier that the parasitism of sandal is a feature to reckon with. It is, therefore, natural to expect that the character of the associated host plant should primarily contribute to the weakness or otherwise of the parasite. In fact, the growth of sandal varies with different hosts. Leguminous plants, such as *Acacias*, pongamia, etc., help to a more rapid growth of sandal. Even among the legumes, there appears to be a difference in the supply of food materials as evinced by the growth and development of the parasite. These host plants aid sandal by a large supply of nitrogen in addition to minerals. Moreover, the rate of attack by sandal roots varies with diverse species. In the field, one encounters innumerable haustorial attachments on the roots of lantana, pongamia, and others, while many other species are not so heavily invaded. What conditions determine the life and duration of haustoria are still obscure.

With reference to spike disease, some of these host plants act adversely, by weakening sandal. Thus, the incubation period is considerably diminished when sandal growing in association with *Acacia* sp., etc., is grafted with diseased tissues. Only very few species really favor it with a capacity to resist disease, as judged by the incubation time. Most of these experiments have been carried out in pots, and demand considerable care in interpreting the same, when a perennial such as sandal is concerned. Until now no one single species can be claimed to have offered complete immunity against infection for sandal.

But, in a general way, it may be said that in most of the areas where spike has had a sway, lantana is predominantly present. Even though stray plots and patches can be discovered with spike and no lantana, the more common observation relates to a higher incidence of disease in lantana-infested areas. The rôle of lantana in relation to sandal spike already has been referred to by the author (16). The more direct test should confine itself to elimination of this weed and then compute the rate of spread with adequate controls. Experiments on the removal of the exotic through chemical treatments have been successfully tried.[1] This process is more economical, too. Further reference to this problem will be made elsewhere.

Although no correlation has been possible between physiological factors and spike incidence, there is one observation that deserves close examination. This relates to the fact that a predisposed tree or one that is likely to get infected in the coming season, produces an abnormally large quantity of flowers. The composition of the leaves at this stage is marked by a high lime

[1] Varadaraja Iyengar, A. V. Thesis, degree of Doctor of Science, Madras Univ. 1934.

content and a significantly low value for nitrogen. These observations are in striking contrast with what has been recorded by the writer in the case of diseased ones in which the reproductive phase is completely inhibited.

CONCLUSION

In the résumé presented here, it has been indicated that the infectious nature of spike necessitates prompt action in eliminating all possible factors that contribute to the spread of the disease in the forest. Since there is no certainty as to whether the attack may be mild or virulent, or as to the area that may next be affected, this problem assumes greater importance. The most serious item for attention is the diseased plant itself. The significance of this aspect has been strikingly brought out by the fact that this is the center of infection, no matter what the method of transmission, whether through haustoria, rootsuckers, or insects. Therefore, attention has been drawn to its economically accomplished elimination through application of toxic chemicals, whereby alone, the spread of disease has been and can be considerably checked. This procedure is now adopted in Madras and Coorg. A few areas with highly virulent stage of spike must be dealt with in the above manner, in order to keep the spread of infection under control.

Our knowledge of the carrier of infection is still obscure. Even here, removal of the diseased plant simplifies the problem. The possibility of the carrier of infection transmitting the disease from other alternate hosts of spike has not been lost sight of, but seems to be of less importance.

Sufficient attention has been drawn to the necessity of proper diagnosis of the disease under field conditions and the phenomenon of masking of spike. Much yet remains to be done on the factors of susceptibility and resistance of this plant parasite to the invasion of one of its most serious diseases. A good deal of improvement on the growth of sandal can be realized through eradication of lantana, a plant that has slowly permeated even some of the dense forests of the Deccan plateau.

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SOME EFFECTS OF RUST INFECTION ON THE DRY WEIGHT OF HOST TISSUES[1]

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INTRODUCTION

In the literature dealing with the effect of rust fungi on their hosts, no satisfactory data have been found on the effect of rust infection on the dry weight of infected tissues. Where hypertrophy of the host occurs in response to rust infection, a local increase in dry weight is to be inferred; in the absence of hypertrophy, the weight of the mycelium might be expected to cause an increase in dry weight in such compatible host-parasite relations as exist in most rust infections (1, 2). The previous observation of the senior writer that rust infection increases the dry weight of clover leaflets floated on sucrose solution in the dark (7) is not a reliable index to the effect of rust on leaves attached to the growing plant.

The present study, principally with the uredial stages of *Uromyces phaseoli* (Pers.) Wint. on bean, *Phaseolus vulgaris* L., and *Puccinia helianthi* Schw. on sunflower, *Helianthus annuus* L.—two rusts that affect only or principally the leaves of their hosts, and that cause no obvious hypertrophy —is an attempt to analyze the nature of the effect of these fungi on the dry weights of their hosts.

The term "increase," as here used, means the greater dry weight, or green weight, of infected plant tissues in comparison with control noninfected tissues of similar area and age, grown under the same environment, and harvested simultaneously; and "decrease" means the lowered weight of tissues due to rust infection.

[1] The assistance of nontechnical employees of the Works Progress Administration is acknowledged.

THE DRY WEIGHT OF THE RUST-INFECTED REGIONS OF LEAVES

The effect of rust infection on the dry weight of leaf tissues of 11 hosts was studied by comparing the dry weight of discs of rust-infected tissue with the weight of similarly located discs of healthy tissue from the opposite halves of the same leaves. The discs of the first 3 collections listed in table 1 were 6.7 mm. in diameter, the remainder were 3 mm. in diameter. The use of smaller discs made possible a better separation of infected and noninfected tissues. From field collections it was difficult to secure closely comparable paired units in which all the infected samples were uniformly infected and all the control samples were entirely free of infection; therefore, an estimate is recorded of the percentage of the area infected in the different samples. The samples were dried to constant weight at 70° C. and weighed. The difference in weight between the paired rusted and control samples was expressed as a percentage of the weight of the controls.

In table 1 is shown the effect of various rusts on the dry weight of infected leaf tissues of their respective hosts, as measured by the above method. With the exception of the last 2 collections that were from greenhouses, all material listed was collected in the field, and in all cases the duration of the infection was not known. The material was collected during the day and sample discs were cut several hours later. As will be shown later (Table 4), the time of day of collecting samples may be an important factor in the results obtained. For each dry-weight determination there were 3 to 15 discs in each member of a paired sample, and 3 to 17 pairs of samples for each value listed.

The accuracy of this method of studying dry-weight changes may be illustrated by the later results with beans. With 60 pairs of leaf samples of five 3-mm. discs each (Table 4), the average deviation of a single determination of dry weight of healthy tissue per unit area from the mean of 10 determinations was 11.9 per cent of the mean value, and the average deviation of a single determination of dry-weight change attributable to rust infection from the mean of 10 determinations was 28.2 per cent of the mean value.

With the exception of *Puccinia canaliculata* on *Xanthium* sp., which was in the aecial stage, all rust collections listed were in the uredial stage, and in no case, with the exception of *Xanthium,* was there any apparent hypertrophy of the host tissue because of rust infection.

The results given in table 1 indicate that rust-infected leaf tissues generally contain considerably more dry matter than comparable healthy tissues. The tabulated results with *Melampsora* on *Populus* are an exception to this rule, but these results may be atypical.

THE DRY WEIGHT OF ENTIRE INFECTED SUNFLOWER LEAVES

The dry weights of infected and noninfected paired primary leaves on the same plant, and the dry weights of leaves of infected and noninfected paired plants, and paired pots of plants were compared (Table 2), using

TABLE 1.—*The effect of rust infection on the local dry weight of leaf tissue of different hosts*

Date	Pathogen and host	Number of paired samples	Estimated percentage area of samples visibly infected with rust		Dry weight of samples, per square decimeter		Percentage difference in dry weight of rusted tissue compared with healthy
			Control	Rusted	Control	Rusted	
			Per cent	*Per cent*	*Mg.*	*Mg.*	*Per cent*
Sept. 12, 1934	*Uromyces betae* on *Beta vulgaris*	5	5	40	955	1219	+ 27.7
" 15, "	*Tranzschelia punctata* on *Prunus domestica*	3	5	15	840	853	+ 1.5
" 17, "	*Melampsora* sp. on *Populus* sp. . .	5	10	30	1264	1225	− 3.1
Oct. 1, 1934	*Uromyces phaseoli* on *Phaseolus vulgaris*	5	5	40	815	968	+ 18.8
" 11, "	*Puccinia canaliculata* on *Cyperus esculentus*	5	0	80	519	1090	+110.0
" 11, "	*Puccinia canaliculata* on *Xanthium* sp.	5	0	100	1450	2488	+ 71.5
" 11, "	*Puccinia polygoni-amphibii* on *Polygonum acre*	5	5	50	1058	1144	+ 8.1
" 11, "	*Puccinia iridis* on *Iris* sp.	5	5	80	1180	1498	+ 26.9
Nov. 26, 1937	*Puccinia antirrhini* on *Antirrhinum majus*	9	5	100	1346	1809	+ 34.4
Dec. 3, "	*Phragmidium* sp. on *Rosa* sp.	17	2	40	504	534	+ 10.6
" 9, "	*Puccinia helianthi* on *Helianthus annuus*	12	0	70	227	273	+ 20.5

greenhouse-grown sunflowers. The noninfected leaf of the pair on a single plant was protected during inoculation by a previous spraying with 1 per cent lime-sulphur + 0.1 per cent sodium oleyl sulphate, or by covering with a glycine bag. The fungicide was found to increase the dry weight of the treated leaves by about 2 per cent, an amount so small that it had little effect on the weights being measured. In all cases where leaves on one set of plants were compared with check leaves on another set, the pairing was done before inoculation.

TABLE 2.—*Effect of rust infection on the dry weight of sunflower leaves 1937*

Units compared	Date of test	Infection period	Paired samples	Increase in dry weight of rusted leaves over paired control leaves[a]
		Days	*No. of*	*Per cent*
Twin leaves	March 19[b]	15	13	28.8 ± 6.5
	" 30[b]	10	13	11.3 ± 2.3
	" 30[b]	14	14	21.7 ± 3.2
	Nov. 27	12	16	12.3 ± 3.4
	" 27	26	5	32.1 ± 9.7[c]
Paired pots	April 12	11	5	10.9 ± 2.4
	" 23	15	10	10.4 ± 6.9
Paired plants	Aug. 24	15	12	17.9 ± 7.6
	" 24	28	8	27.9 ± 17.8
	Oct. 19	11	10	15.2 ± 7.3
	" 19	20	10	13.5 ± 9.7
	" 19	28	10	2.0 ± 4.2[c]
	Nov. 12	14	9	7.3 ± 9.7
	" 12	21	8	45.9 ± 19.1

[a] Mean and standard error of mean.
[b] Control leaves were protected by spraying with 1 per cent lime sulphur + 0.1 per cent sodium oleyl sulphate. In all other cases controls were protected by means of glycine bags, if necessary.
[c] Rusted leaves dying or dead.

Potted plants were inoculated by dusting spores of *Puccinia helianthi* on the upper surface of the leaves, atomizing them with water, and holding them overnight in moist chambers. This method does not produce such heavy infection as do inoculations on the lower leaf surface, but the infection was more uniform.

The severity of infection was not measured in most tests, but, in the test of November 12, the 44 leaves varied from 10.3 to 25.2 sq. cm. in area and the number of primary pustules per square centimeter varied from 25 to 87. This is heavier than the infection in most tests.

This method of making comparisons between opposite twin leaves, one of which is infected and the other not, is believed to be very accurate because twin sunflower leaves and the twin leaves of many other plants (4) are very closely comparable. With 22 random pairs of primary sunflower leaves from plants of the same age, the average deviation of a single leaf from the mean of a pair of leaves was only 3.6 per cent of the mean value for area and 3.9 per cent for dry weight, while for the same leaves the average devia-

tion of a single leaf from the mean of all leaves was 19.7 per cent for area and 24.5 per cent for dry weight. A part of the measured deviation in the paired samples was probably caused by errors in the rapid planimeter measurements of leaf area and the weighing of the leaves to only the nearest milligram.

The results of 14 tests with a total of 143 paired samples (Table 2), show that rust-infected leaves were always greater in dry weight than paired control leaves, the measured difference varying from 2 to 46 per cent. The reason for the great range of difference is partly the difference in infection period. In 4 cases (March 30, August 24, November 12, and November 27) 2 tests with different infection periods were started on the same day, and in each case the difference in dry weight was much greater with the longer infection period. The test of October 19, however, is an exception, for here the difference due to rust was less with longer infection periods. In this test, however, paired plants were compared and error is much greater than with paired leaves. It would seem quite possible that the effect of rust infection might be entirely different when only 1 leaf of a pair is infected than when both are infected. The present results, though the data are inadequate, would indicate, however, that the effect of rust on dry weight is similar whether comparisons are made between 2 leaves of a single pair, between paired plants, or between paired pots of plants.

Rust-infected leaves were also greater in green weight, and in percentage dry weight of the original green weight, than healthy leaves. In 3 tests (the same as the first 3 tests of table 2) rusted leaves were greater in green weight by 8.5 ± 5.3 per cent, 6.1 ± 2.7 per cent, and 11.9 ± 2.3 per cent, respectively, than healthy control leaves. In these same tests the percentage dry matter of healthy leaves was 13.2, 14.9, and 12.9, respectively; and of rusted leaves 15.5, 15.7, and 13.9. The percentage dry matter in the rusted leaves was greater by 14.7 ± 2.45 per cent in the 3 tests comprising 40 pairs of determinations.

EFFECT OF RUST INFECTION ON THE GROWTH OF SUNFLOWER AND BEAN PLANTS

The effect of severe rust infection in reducing the top and seed yield of many plants (2), and, specifically, that of sunflowers (3), is well known. Data on the effect of rust infection on the dry weight of leaves, stems, and new growth of sunflowers in one representative test are given in table 3. In this test, a member of each paired sample was inoculated when the plants had 1 pair of well-developed true leaves and the growth distal to this pair of primary leaves was removed. The growth that occurred distal to the primary leaves after inoculation is considered the new growth. Eleven days after inoculation the weight of the primary leaves of inoculated plants was greater by 15 per cent, the weight of the stems of inoculated plants was less by 12 per cent and the weight of the entire inoculated plants was less by 3 per cent than the weights of paired control plants. By 28 days after

TABLE 3.—*Effect of rust infection on the dry weight of sunflower plants with varying periods of infection*

Plant part	Condition of plants	Mean dry weight in mg. per plant at varying times after inoculation			Difference in percentage[a] mean dry weight of rusted plants compared with controls at varying times after inoculation		
		11 days	20 days	28 days	11 days	20 days	28 days
Total plant, exclusive of roots	Control	128	302	411			
	Rusted	124	256	240	− 3.4 ± 8.8	−13.9 ± 6.8	−41.1 ± 6.1
Primary leaves only	Control	44	73	78			
	Rusted	51	83	80	+15.2 ± 7.3	+13.5 ± 9.7	+ 2.0 ± 4.1
Stem only	Control	84	199	259			
	Rusted	74	148	138	−12.4 ± 10.2	−26.4 ± 9.9	−46.9 ± 4.6
New growth only	Control	0	30	84			
	Rusted	0[b]	7.7	3.0	[b]	−77.8 ± 42.7	−96.4 ± 17.9

[a] Mean and standard error of mean for 10 paired samples.
[b] The new growth here was so small that no measurements were made.

inoculation the weight of the primary leaves of inoculated plants was greater by 2 per cent, the weight of the stems of inoculated plants was less by 47 per cent, the weight of the new growth of inoculated plants was less by 96 per cent, and the weight of the entire inoculated plants was less by 41 per cent than the weights of paired control plants. The results at 20 days after inoculation were intermediate. Confirmatory results were secured in 4 other tests, in 2 of which the growth distal to the inoculated primary leaves was not removed at inoculation. In these 2 tests the reduction in the weight of new growth (growth distal to inoculated leaves) due to rust infection was only 12 and 15 per cent at 14 and 15 days after inoculation, respectively, much less than when the new growth was removed at inoculation.

In one test on January 26, 1938, in which the primary leaves of paired young greenhouse Pinto bean plants were inoculated and the growing points were not removed, the dry weight of the entire rusted plants was 15.3 per cent less, and the weight of the infected primary leaves 15.8 per cent greater than the healthy plants 10 days after inoculation. Twenty-one days after inoculation the dry weight of entire plants was 29.6 per cent less and the dry weight of the primary leaves 47.9 per cent greater than the healthy plants. On the basis of this one test the response of bean plants to rust appears to be similar to that of sunflowers.

THE EFFECT OF RUST INFECTION ON THE DIURNAL CHANGES IN DRY-MATTER CONTENT OF BEAN AND SUNFLOWER LEAVES

In tests begun January 3, 1938, 18 days after Pinto beans with one pair of primary leaves had been inoculated with rust, 3-mm. discs were cut from infected and noninfected portions of the same leaves at contrasting times of the day, and weighed (Table 4). To secure closely comparable samples, the afternoon samples were taken from the opposite halves of the same leaves used for the morning samples. In one series (January 3) the afternoon samples were taken first, and in the other 2 series the morning samples were taken first. The rust pustules were large, mostly single, and about 80 per cent of each disc recorded as rusted consisted of infected tissue. The afternoon samples were taken at about 3 p.m. with the hope that they would represent the high phase of the diurnal change in dry weight of leaves (6). The morning samples were taken at 8 a.m. after the plants had been held overnight in a dark chamber.

In these 3 tests, the local increase in dry weight as a result of rust infection varied from 50 to 90 per cent and was always greater in the samples taken at 8 a.m. The diurnal fluctuation was much greater in the noninfected tissue, where it ranged from 15 to 29 per cent of the 8 a.m. value, than in the rusted tissue, where it was about 6 per cent of the 8 a.m. value. Although the differences in dry weight due to time of sampling are not statistically significant for the rusted samples as calculated, the uniformity of the 3 independent determinations must be considered as adding to the significance of the differences.

TABLE 4.—*The effect of rust infection on the diurnal change in the dry matter of bean leaves, 1938*

Series No. and date	Condition of samples	Mean dry weight of leaf samples		Increase of rusted over healthy[a]		Increase of 3 p.m. samples over 8 a.m. samples[b]
		8 a.m.	3 p.m.	8 a.m.	3 p.m.	
		mg. per dcm.^{2}	*mg. per dcm.*^{2}	*Per cent*	*Per cent*	*Per cent*
1. January 3	Healthy	214	277			29.5 ± 6.1
	Rusted	393	416	83.7 ± 5.8	50.2 ± 5.0	5.9 ± 5.1
2. January 5	Healthy	181	220			21.5 ± 6.7
	Rusted	345	367	90.6 ± 9.2	66.8 ± 12.4	6.4 ± 7.6
3. January 6	Healthy	229	263			14.9 ± 5.6
	Rusted	417	442	82.2 ± 6.9	68.0 ± 10.5	6.0 ± 7.2

[a] Mean and standard error of mean for 10 pairs of samples.

[b] Difference and standard error of difference. In this case the standard error is of the difference of the means of independent groups, as there was no pairing of the separate values; for this reason the error is high.

The effect of sunflower rust on the diurnal fluctuation in the dry-matter content of greenhouse sunflower leaves was determined in one test on December 1, 1934, 8 days after inoculation. An estimated 15 per cent of the area of the rusted samples was infected, and the control samples were free of rust. Six paired samples were collected for each time of sampling. The dry weight per square decimeter of leaf was 271 and 290 mg., respectively, for the healthy and rusted paired samples at 5 a.m. and 287 and 297 mg., respectively, at 2 p.m. The increase in dry weight due to rust infection was therefore 3.45 per cent at 2 p.m. and 6.78 per cent at 5 a.m. and the diurnal increase in dry matter (2 p.m. samples as compared to 5 a.m.) was 5.42 per cent for healthy areas and 2.38 per cent for rusted areas.

DISCUSSION

The principal contribution of the present study appears to be the information on the effect of rust infection in increasing the dry weight of infected leaf tissues. The increases in dry weight of leaves due to rust infection varied from -3.1 per cent to $+110$ per cent (Table 1), and most of the low values are apparently due to the low infection in the material studied. From the data of tables 1 and 4 the mean increase in dry weight of leaves due to rust infection was 45.2 ± 8.5 per cent for the 17 paired collections from 11 hosts. If, however, allowance is made for the low percentage area of the samples visibly infected, by assuming the increase in dry weight to be proportional to the percentage area of the samples visibly infected the average increase in dry weight was 61.2 ± 9.9 per cent. The fact that 7 of these 17 samples were bean rust tends to increase the mean.

There is great variation in the effect of a given rust on the dry weight of its host's leaves. This is well illustrated by sunflower rust, with which the measured increase varied from 2 per cent to 46 per cent (Table 2), and by bean rust, in which the measured increase varied from 19 per cent (Table 1) to 91 per cent (Table 4). Three causes of this variation have already been mentioned. The increase is greater with increased severity of infection (Table 1), is greater with increased infection period (Table 2), and is greater for samples taken in the morning than for those taken in the afternoon (Table 4). Several other factors such as environmental conditions would probably also affect the results.

The causes of the increase in dry weight of leaf tissues due to rust infection have not been determined in this study, but an explanation may be suggested by the results obtained. From the data of table 3 and from other data in the text, it is apparent that the increase in dry weight of infected leaves of sunflowers and beans is associated with a decrease in the dry weight of the noninfected stems and new growth, and that the decrease in the weight of stems and new growth is greater than the increase in the weight of the infected leaves. In young plants with 1 pair of primary leaves the new growth and the increase in the weight of the stems with time is due principally to the organic materials photosynthesized in the primary leaves

and translocated to these other parts. Rust infection, therefore, has apparently decreased the translocation of food from the infected leaves. The reasons for this decrease in translocation are probably threefold. First, rust infection probably interferes with the mechanism of photosynthesis so that less total food is formed. The writer has no satisfactory data on this point, but the data of table 4 relative to diurnal variation in dry matter content of bean leaves would tend to support it. Secondly, rust infection greatly increases the respiration of infected tissues (7). This would dissipate *in situ* a considerable portion of the carbohydrates photosynthesized in the leaves. Thirdly, rust infection causes a fixation of considerable dry weight in the infected leaves. It would, therefore, appear that the rust fungi may be nourished principally by the labile organic materials photosynthesized in the leaves, and translocated from them in the case of healthy leaves. That rust fungi cannot be well nourished by the more stable organic materials of leaf tissues is indicated by the paucity of rust development on plants in the dark, unless these plants are supplied with readily available carbohydrates. A somewhat similar line of reasoning has been developed by Mains (5).

SUMMARY

In 10 out of 11 collections of different rusts (on beet, prune, bean, *Cyperus, Polygonum, Xanthium, Iris,* snapdragon, rose, and sunflower) the local dry weight per unit area was greater in rusted than in healthy leaf areas. The differences ranged from – 3.0 to + 110.0 per cent, with an average of + 33 per cent. The rust on poplar proved to be an exception, as measured, but the results may be atypical.

In 14 tests with paired sunflower leaves or plants, the dry weight of the entire infected leaves was from 2 to 46 per cent greater than the dry weight of the paired controls. In 3 tests the green-weight of infected leaves averaged 9 per cent more than that of noninfected leaves and the percentage of dry matter of infected leaves averaged 15 per cent more than that in noninfected leaves.

The reduction in dry-weight yield of entire sunflower and bean plants as a result of rust infection of the primary leaves was associated with a higher yield of infected leaves than of healthy leaves, a lower yield of stems from infected plants than those from healthy plants, and a lower yield of the new growth of infected plants than of healthy plants.

In 3 tests with beans and in 1 test with sunflowers, rust infection decreased the diurnal fluctuation in the dry weight of the leaves.

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SPECIES OF EUTYPELLA AND SCHIZOXYLON ASSOCIATED WITH CANKERS OF MAPLE

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INTRODUCTION

During a forest disease survey[1] in the Lake States in the fall of 1935, a study of fungi associated with cankers on sugar maple, *Acer saccharum* Marshall, and red maple, *A. rubrum* Linn., led to the recognition of two distinct cankers, herein referred to as Eutypella[2] and Schizoxylon. As these cankers seemed to be undescribed, preliminary work was done to determine their prevalence and distribution and to establish the pathogenicity of the associated fungi. This work was necessary to ascertain the importance of these cankers in the forest stand and also to determine whether control measures were advisable. The purpose of this paper is to record the characteristics of the cankers, to present data concerning their occurrence, and to describe the fungi associated with them and believed to be their cause.

EUTYPELLA CANKER

Distribution and Prevalence

A canker, with which a species of *Eutypella* was consistently associated, was found on sugar and red maples throughout all the national forests of the Lake States. It was most abundant in the Chequamegon, Nicolet, Ottawa, and Upper Michigan National Forests. The fact that it also has been collected by one of the writers and others[3] on cankered sugar and red maples outside of the Lake States indicates that it occurs over an extensive area.

[1] Lorenz, R. C. and Christensen, Clyde M. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. October 7, 1937. (Mimeographed.)

[2] In 1933 Frank Kaufert, Pathologist, Pest Control Research Section, Du Pont Laboratories, observed and collected this type of canker on sugar maple in Itasca State Park, Minnesota, but no determination of the associated fungus was made. (Unpublished report.)

[3] Specimens of this canker and the associated *Eutypella* on sugar maple were collected by other workers in the following localities and sent to the writers for identification: Carrol, New Hampshire, May 12, 1933, by J. R. Hansbrough and T. J. Grant; West Burke, Vermont, Oct. 11, 1933, by D. S. Welch (No. 1343); Seventh Lake, New York, Aug. 25, 1934, by D. S. Welch and F. C. Stewart (No. 1342); Carrol, New Hampshire, May, 1935, by T. J. Grant; Gale River, New Hampshire, Nov., 1937, and Danby, Vermont, May, 1938, by W. A. Campbell.

Cankers believed to be caused by this species of *Eutypella* were found much less frequently on red than on sugar maple. Sample plots 1/10 acre in area indicated that 7 per cent of the sugar maples were affected on the Chequamegon National Forest and that most of the diseased trees were of the suppressed and intermediate crown classes. In the mature stand of northern hardwoods near the James Lake Camp on the Ottawa National Forest, about 2 sugar-maple trees per acre were cankered. The canker was common on sugar maple in the vicinity of the Argonne ranger station on the Nicolet National Forest. On the Dukes Experimental Forest, in upper Michigan, the disease was common on sugar maple of all age and crown classes and appeared to be more prevalent there on older trees than elsewhere. Only an occasional cankered tree was found in the Cadillac ranger district of the Manistee National Forest, where northern hardwoods are common. From 20 to 30 cankered trees per acre occurred in a pure stand of young sugar maple near Cloquet, Minnesota.

Economic Importance

Trees over 5 inches diameter breast height are seldom killed by this disease, but smaller trees, especially those suppressed in the 1- to 3-inch diameter classes, often are killed. The death of some of these trees is probably due to the combined effect of the fungus and suppression, but small trees not suppressed may be killed. Cankers persist on the larger trees for many years and occasionally reach a length of 5 feet (Fig. 2, C). Such trees are liable to breakage at the canker.

At present it seems likely that this disease is of importance only in those areas that contain a high proportion of sugar maple. It does not appear to be doing much damage in the well-mixed stands of northern hardwoods, which are comprised chiefly of birch, beech, maple, and hemlock.

Description of the Canker

Eutypella cankers are characterized in general by firmly attached bark, heavy white to buff mycelial fans under the bark at margins, broad, slightly raised concentric rings of callus tissue, and the presence of long-beak perithecia with their black, sulcate ostioles protruding above the bark in the centers of old cankers. They are usually similar in appearance to Strumella cankers.

The bark becomes tightly attached to the wood by the dense mycelial mat. This mycelial layer (Fig. 1, A) is at first white to buff, consisting of radiating strands of hyphae closely matted together, but it gradually darkens and coalesces with the bark and wood. The heavy mycelial fan development, which is always present, is the most characteristic feature of this canker.

The annual extension of cankered areas is usually uniform, averaging about ½ inch (Fig. 1, A). However, this extension is occasionally checked suddenly by some unknown cause, around either the entire margin or on only a portion of it. Such sudden cessation of the extension gives the canker a

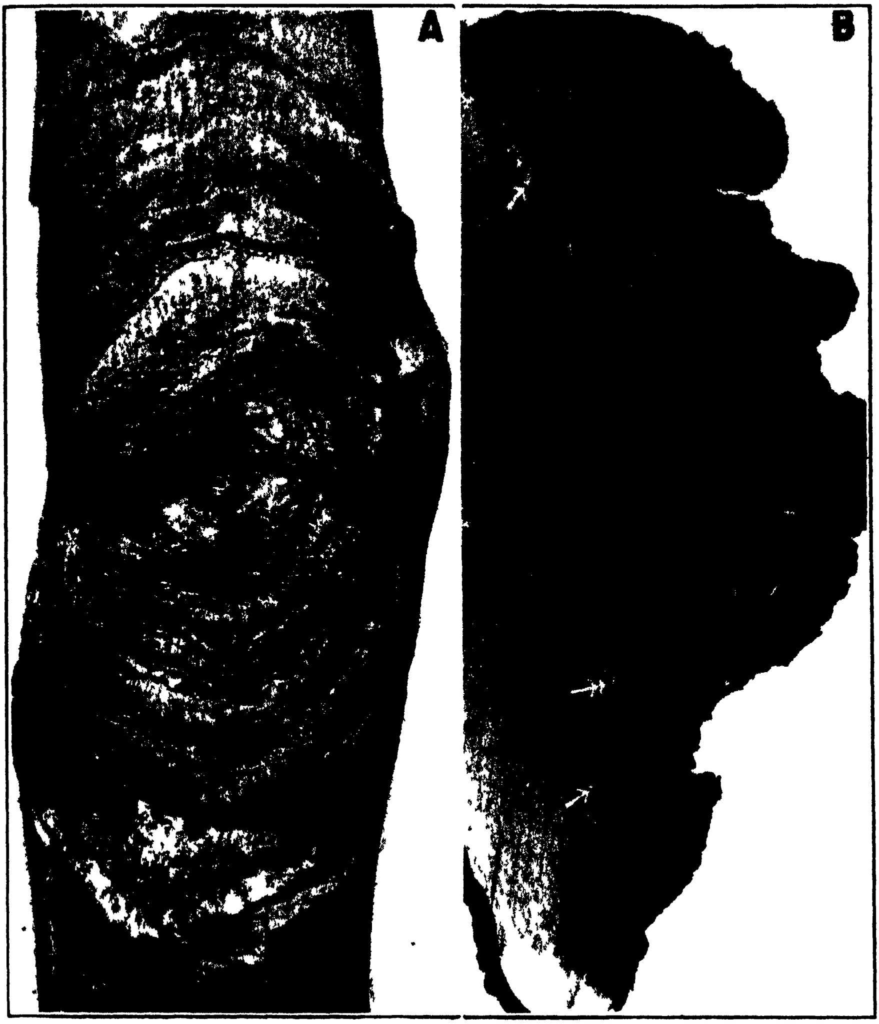

FIG. 1. A. Young Eutypella canker on 3.5-inch trunk of red maple. Perithecia are abundant in center of this canker. Note mycelial fans at lower margin of canker where bark has been removed. B. Cross section of old canker on 10 inch sugar maple. Arrows show white mycelium of *Eutypella* growing out along margin of diseased and healthy wood. Decay in center of this section was caused by *Polyporus glomeratus*. Photographs by M. L. F. Foubert.

rough or irregular appearance, whereas, normally, it would be relatively uniform, and as a tree bearing such a canker becomes older, considerable distortion of the stem results (Fig. 2, A to C).

Eutypella penetrates deeply into the sapwood beneath the canker and usually extends beyond the center of the tree. It also grows slowly upward and downward, forming a central column of discolored wood. Infected wood near the center of the canker finally becomes brown and brittle in appearance and seems to be slightly decayed A single species of *Eutypella*

has been consistently isolated from this discolored wood—both from under the center of cankers and from near the margins of the discoloration. This ability of the fungus to penetrate the wood probably prevents the cankers from becoming permanently inactive.

Older cankers usually contain perithecia imbedded in the outer bark of the central portion and their necks protrude slightly above the surface, giving the bark a blackened appearance. Perithecia have not been observed to develop nearer than the fourth or fifth annual callus ring from the margin of cankers, and frequently they are confined to only a small central portion

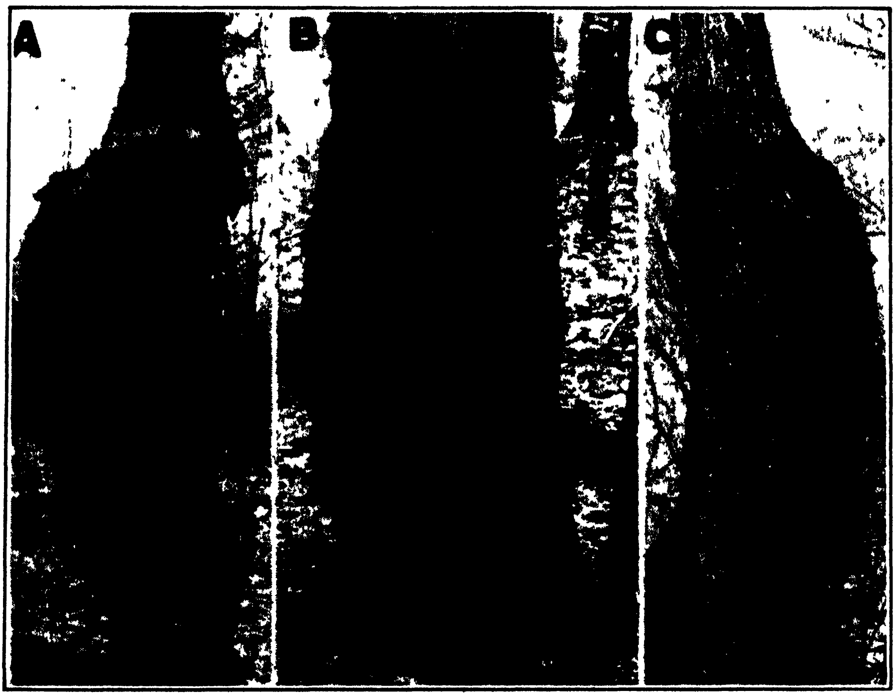

FIG. 2. Eutypella cankers on sugar maple showing manner in which the trunks are deformed. A. Cankered 6-inch stem. B. Large canker at base of trunk. C. Canker 5 feet in length and about 10 feet up from base of 12-inch stem.

of large cankers. It seems probable that fruiting depends on thorough invasion of the wood, which is apt to occur sooner under the central portion. Cankers collected at various times indicate that mature perithecia persist throughout the year. That is, whenever perithecia were present mature viable spores could be obtained from most of them.

Initial infection apparently occurs when the tree is young, since no young infections ever were found on older trees; but, once a tree is infected, development continues for many years. Cankers usually occur at heights of 2 to 8 feet aboveground, but have been observed in a few cases at ground line or at a height of 30 feet. All observed cankers have been on the main stems, but dead branch stubs were usually present at their centers. Usually, there is only one canker on a tree.

Inoculations

In the summer of 1936, Lorenz and Kaufert inoculated numerous sugar-maple trees with *Eutypella*. In the spring of 1937, 3 of these trees were cut and the fungus reisolated from the host tissue surrounding the point of inoculation in each case. A small area of cambium around the inoculation injuries was dead, but insufficient time had elapsed for typical canker formation to be expected. Check injuries on these same trees gave no *Eutypella* in culture when isolation attempts were made. The information is, however, considered insufficient for proof of parasitism.

Associated Fungus

Isolation Results: There were no fruiting bodies on the first specimen of this disease, which was sent to the Division of Forest Pathology in the fall of 1935; but numerous isolates from both the cambium at the margin of the canker and the discolored wood beneath uniformly consisted of a white mycelium, which, in 4 to 6 weeks, bore an abundance of *Libertella* spores. Many older cankers collected later contained mature perithecia of a species of *Eutypella,* the ascospore cultures of which were similar in every respect to the cultures isolated from the host tissue.

Isolations were later made from the margins of a number of cankers of this type and from discolored wood beneath the cankers and cultures of the above-mentioned fungus were consistently obtained. Even from the brittle brown wood frequently present under the center of the canker, the fungus was usually isolated in pure culture. This would indicate that when this species of *Eutypella* is well-established in the wood, other fungi usually are excluded. This characteristic also is shown by the fact that, even where insects have tunnelled through the wood, pure cultures of it usually are obtained. Figure 1, B shows a cross section of a large canker on an old sugar maple that contained decay (*Polyporus glomeratus* Pk.) in the central portion. It will be seen that a black line had formed around the decay on the side next to the canker. From inside this black line, only the decay fungus was obtained in culture, while from the brown, brittle wood on the outside of the black line, only *Eutypella* was isolated. The same cross section shows white mycelium of *Eutypella* growing out along the line between the brown discoloration and the living sapwood.

Cultural Characteristics of the Eutypella: The *Eutypella* grows readily on various culture media such as malt, potato dextrose, and corn meal, but a 2.5 per cent malt (Trommers) agar medium was used throughout this study. The mycelium is fairly dense and remains white (Fig. 4, A and B), except in older test-tube cultures, where it may develop patches of light buff to cream color. The color is usually present at the lower margin of the slanted agar, where the mycelium grows between the glass side of the tube and the agar. It is in such positions that spores usually are produced. The long, curved spores (Fig. 5, H) are not formed in a pycnidium but occur in great abundance on an indistinct layer in the mycelium. The cultures usually require

a 4- to 6-week incubation period at room temperature (25° C.) for spore production. Perithecia have not been obtained in culture.

As there are a number of closely related genera having conidia similar to those of the form genus *Libertella*, any attempt at identification from cultural characteristics would have to take into consideration species of *Diatrype, Diatrypella, Eutypa, Valsella,* and probably several others, as well as those of *Eutypella*. Cultures of only one other species of *Eutypella* have been available for such comparison, but a number of ascospore cultures of species from related genera have been used, and the preliminary study indicates that identification on the basis of cultural differences might be possible. *Eutypella angulosa* (Nits.) Sacc. from yellow birch, *Betula lutea* Michaux, for instance, grows more rapidly in Petri-dish cultures; and conidia, red in mass, are produced at the top of test-tube slants. Conidia of *E. angulosa* (Fig. 5, I) are also shorter and less curved than those of *Eutypella* from cankered maple. Ascospore cultures of *Eutypa heteracantha* Sacc.[4] from *Ailanthus* develop an abundance of dark mycelium and its conidia (Fig. 5, J) differ slightly from those of the canker fungus. *Diatrype hochelagae* E. & E., which was collected several times on old cankered areas of maple stems during the present studies, grows much more slowly in culture and has shorter less curved conidia, somewhat similar to those of *Eutypella angulosa* as shown in figure 5, I. Numerous other common species of *Diatrype* such as *D. stigma* (Hoffm.) Fr. and *D. virescens* (Schw.) Cke., and *Diatrypella prominens* Howe, which have been grown in culture but not studied critically, are not likely to be confused in culture with this particular maple fungus, although they have somewhat similar conidial stages. Cultural comparisons should eventually be made between the common saprophytic forms reported on maples and the canker form.

Comparison with Other Species of Eutypella on Maple: Although no species of *Eutypella* or *Eutypa* is known to have been reported in the literature as causing or occurring on cankers on maples, several of the described saprophytic species are somewhat similar to the one we have found occurring on cankers. *Eutypella corynostoma* (B. and Rav.) Sacc. and *E. constellata* (B. and C.) E. and E., which occur on maple, have ascospores of about the same size. Also, the general structure and arrangement of perithecia are similar to those on the cankers.

The ascospores from the cankered specimens seem to be consistently darker and less curved than those examined from the above-mentioned saprophytic forms. These spore characters are consistent for all specimens examined, regardless of apparent differences in age of stromata. Another respect in which the canker strain differs is in its heavier stromatic development, which might result from its restricted area of growth throughout its long period of activity. Because of these slight differences and its association with a definite canker, we are not assigning it to any previously named species. Since no extensive monographic study has been published on *Eutypella* and

[4] This and several of the other species referred to were identified by C. L. Shear, formerly principal pathologist, United States Department of Agriculture.

related genera, it has not been possible to make satisfactory comparisons with the many inadequately described species. For convenience of future workers this *Eutypella,* which, so far as is at present known, occurs only on cankers on maple, is named and described as a new species. A type specimen has been deposited in the mycological collections of the Bureau of Plant Industry, Washington, D. C.

Technical Description: **Eutypella** (*Eutypa*) **parasitica,** n. sp. (Fig. 5, F to H).

Pustules usually densely grouped, 3 to 15 mm. in diameter, or coalescent over large areas with closely aggregated ostioles blackening the fissures in the bark; perithecia irregularly crowded together in the slightly altered outer bark (usually in bark closely attached to the wood near center of canker) in groups of 10–40 (sometimes in much greater number), and sometimes imbedded in wood of the central branch stub, occasionally circumscribed by inconspicuous black line, black, large 0.6 to 0.9 mm. diameter; necks long, extending slightly beyond surface of the bark or, when in deep fissures, long and protruding toward the light, cylindric, sulcate at the ostiole; asci small, stipitate, spore-bearing part 32–40 × 6–7 μ, stipe 10–40 × 1.5 μ; ascospores very slightly curved, irregularly 2- or-more seriate, dark (smoky) brown, slightly attenuated at ends, 8–11 × 2–2.3 μ; conidia (found in culture only) long, slender, pointed at the ends, 26–34 × 1 μ, curved (mostly U-shape), hyaline, only slightly buff-color or hyaline in mass.

Growth rate[5] in culture, about 20 mm. in 7 days. Constant temperature studies show good growth at 20° to 35° C., no growth or only a trace at 40° C.

Pustulis orbicularibus gregariis, 3–15 mm. diam. interdum majoribus, in corticali immerso, ostiolis erumpentibus, peritheciis inordinatis congestis, interdum in ligno immersis, 10–40 vel pluribus in stromate singulo, nigris, subglobosis, magnis, 0.6–0.9 mm. diam.; ostiolis longissime exsertis, cylindraceis, sulcatis; ascis cylindrico-clavatis, filiformi-stipitatis, octosporis, p. sp. 32–40 × 6–7 μ, stipite 10–40 × 1.5 μ; ascosporis subdistichis, leniter curvatis, fuscis, 8–11 × 2–2.3 μ; conidiis (in culturis productis) cylindricis, curvulis, utrinque attenuatis, hyalinis, 26–34 × 1 μ.

On cankered maples, *Acer saccharum* Marshall and *A. rubrum* Linn. in Minnesota, Wisconsin, Michigan, New York, New Hampshire, and Vermont. Herbarium specimens 71988 type, 71656, 71001, 71011, 71624, 71110, and 70999.

SCHIZOXYLON CANKER

Distribution, Importance, and Prevalence

Another type of canker, the Schizoxylon canker, was found occasionally on both red and sugar maples in the Chequamegon, Ottawa, Upper Michigan, and Manistee National Forests, but it has not been found outside the Lake States. The information so far obtained indicates that the Schizoxylon canker is of little importance, because it is rare and observed cankers were found usually on suppressed trees less than 3 inches in diameter.

[5] Average radial growth at room temperature of about 25° C.

Description of the Canker

Schizoxylon cankers resemble Nectria cankers. Their annual extension, as indicated by callus folds, is much less than that of those associated with *Eutypella* and results in a smaller, more sunken canker. As the bark remains attached ordinarily for only a few years, exposed wood is present in the center (Fig. 3, A and B). This exposed wood is weathered and often

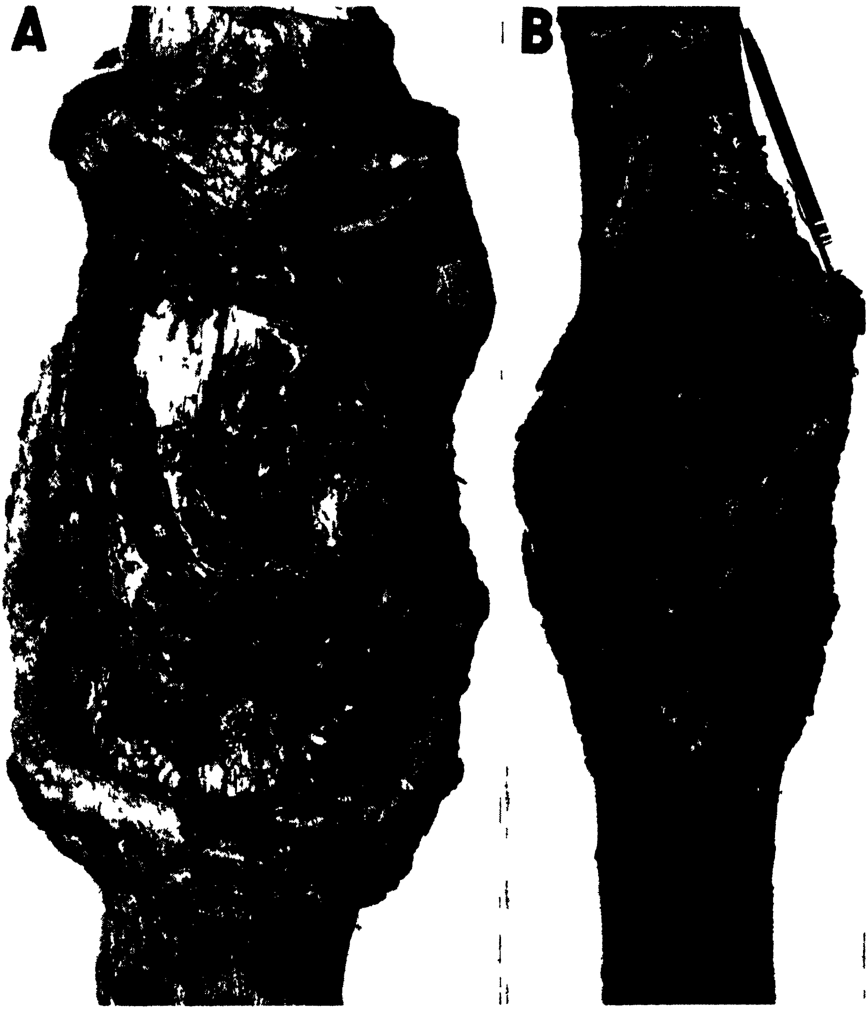

FIG. 3. Schizoxylon cankers. A. Old canker on sugar maple. B. Similar canker on red maple.

partly decayed on the surface. The interior of the infected trunks is discolored, as described for Eutypella-infected trees.

The margins of the cankers where the fungus is active are characterized by the presence of white mycelial fans, which are less conspicuous than those on Eutypella cankers.

The cankers are often irregular, and activity may be entirely checked for a time or may continue in only a small portion of the margin. This activity usually does not cease until the diameter of the cankers is equal to or greater than the diameter of the trunk. It is accompanied by a distortion of the trunks on which it occurs. This is at first manifested by a flattening and later by a protruding margin of thickened callus tissue (Fig. 3, A and B).

The light-grey pycnidia of the fungus can readily be seen sunken in the exposed wood and are often present but less conspicuous in the bark near the margin of the canker.

Isolation Results

Schizoxylon was isolated from the margins of a number of cankers, but the percentage of successful isolations was lower than for the *Eutypella*. It was also frequently isolated from the discolored wood near the surface and occasionally from the internal discoloration, but miscellaneous unidentified fungi were also frequently obtained from the discolored areas. The extremely slow growth of *Schizoxylon* in culture may account for this low percentage of successful isolations in that other wood-inhabiting fungi would suppress it.

Cultures were readily obtained from the pycnospores and were identical with those from wood and bark.

Inoculations

A number of inoculations were made with Schizoxylon at the time when the *Eutypella* inoculations were made. Of three inoculations removed in 1937 reisolations were successful in only one case. In the case of the successful reisolation pycnidia of *Schizoxylon* were abundant on the bark around the point of inoculation. The white mycelial growth had penetrated the bark surrounding the injury, but the cambium was not killed back to any marked extent. The other two inoculations showed no indication of the fungus' having become established and molds and other fungi had grown over the injured area. *Schizoxylon* was not isolated from check injuries on these same trees.

Description of Schizoxylon

In Culture: Schizoxylon grew on various culture media, but 2.5 per cent male agar was used for isolations and growth-rate studies and Difco cornmeal agar for spore-germination work.

This fungus has a characteristic white mycelial mat (Fig. 4, C and D), which, coupled with the slow growth rate, permits easy identification. In fact, it could not be confused with any fungus isolated in the course of these studies. The mycelium is white, but occasional light brown patches occur in older cultures. The mat has a tufted, wavy appearance with numerous globose mycelial bunches that appear to be pycnidial primordia. The margins are thin and irregular.

Fruiting in Nature: When maple cankers were first collected for a study of possible causal organisms it was noticed that a few that resembled

Nectria cankers in general appearance contained light gray pycnidia imbedded in shallow cup-like depressions in the bark and wood. These pycnidia were globose and had no ostioles, but their interiors were filled with a compact mass of small rod-shape spores.

The appearance of the pycnidia suggested that a discomycete, similar to *Stictis*, might be the perfect stage. A search of the literature on *Stictis* and

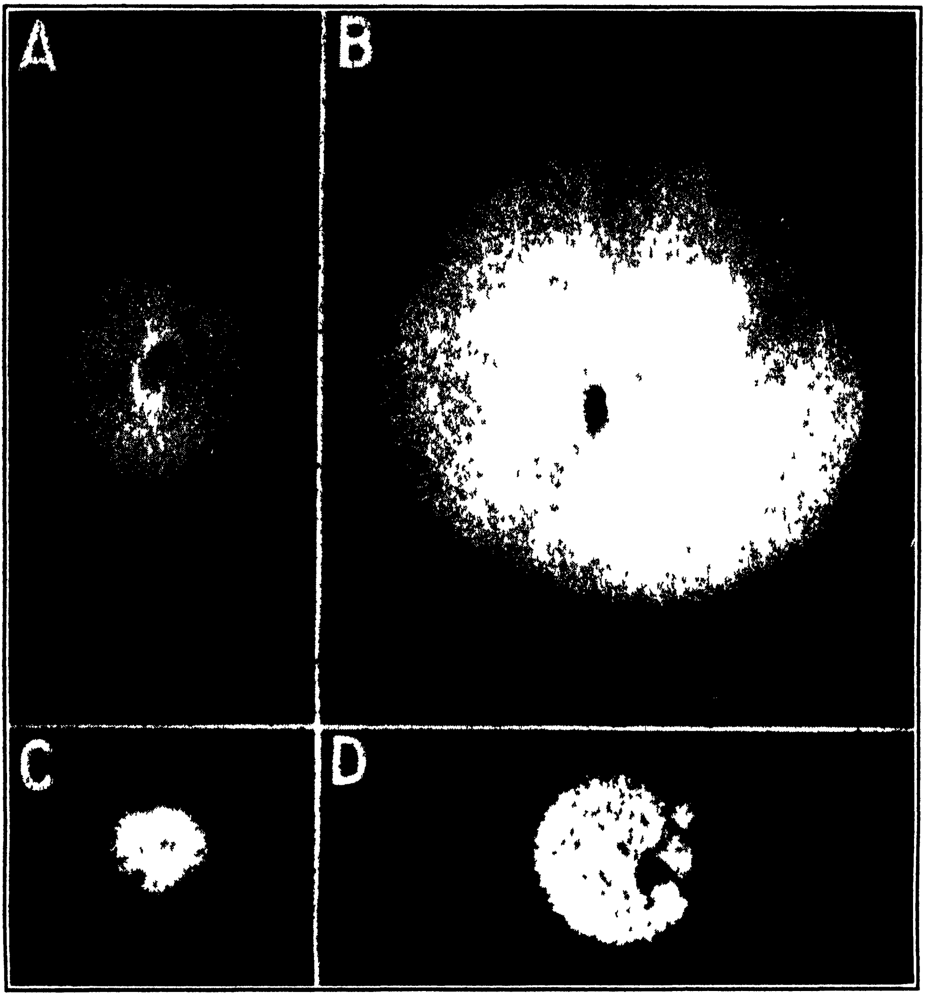

FIG. 4. A and B. 7- and 14 day old cultures of *Eutypella parasitica*. C and D 7 and 14-day old cultures of *Schizoxylon microsporum*.

related genera disclosed only one species, namely *Schizoxylon sepincola* Pers.,[6] reported as having a pycnidial stage. Continued collection of cankers in an attempt to find such a discomycete finally resulted in obtaining one on red maple at Baldwin, Michigan, with a species of *Schizoxylon* fruiting on it. This same canker had typical pycnidia fruiting in association with the

[6] Tulasne. Selecta Fungorum Carpologia. V. 3, page 148. 1865.

apothecia. In appearance the apothecia of this *Schizoxylon* were similar to the pycnidia, except for a small dark opening on top (Fig. 5, K). The interior was dark and hollow in the upper part with a compact layer of asci below. This canker form has no branched paraphyses as are described for *S. sepincola* and its ascospores are smaller. The pycnidial stage is apparently quite similar to that recorded for *S. sepincola.*

Numerous cultures obtained from the small segments of ascospores and from single asci containing germinating spores were similar to the cultures from pycnospores and host tissue.

The *Schizoxylon* found fruiting on the one red-maple canker does not fit the descriptions of any described species.[7] It more nearly fits descriptions of *S. sepincola,* but does not have branched paraphyses and its ascospores are narrower. It is, therefore, described as a new species.

A type specimen has been deposited in the mycological collections of the Bureau of Plant Industry, Washington, D. C.

Technical Description: **Schizoxylon microsporum,** n. sp. (Fig. 4, C and D and Fig. 5, A to E and K).

Apothecia rare, scattered singly or clustered, sunken in the bark or exposed wood, 0.7 to 1 mm. diam., at first covered by a white papillate membrane (black around ostiole) which splits back from the central opening finally flask or cup-shape; asci in a thick layer filling the lower half of the globose fruiting body, 170–200 × 8–12 μ, wall thickened at tip, cylindric, tapering slightly to short pedicel; paraphyses filiform, as long as the asci, septate, not (or only slightly) thickened above, not branched, 1.3 to 1.7 μ diam.; ascospores filiform, almost as long as the asci, multiseptate, 1 μ diam. or less, finally breaking up into short rods or slightly curved segments 2–3 × 1 μ, sometimes longer and septate (4–8 × 1 μ).

Pycnidia common, imbedded in bark or surface of wood, globose 0.6 to 1 mm., white to light gray, similar to apothecia but without papillate opening; spores histogenic, filling pycnidium in compact hyaline mass, small, often slightly curved, 2–5 × 1 μ. Growth rate 5 to 6 mm. in 7 days at ordinary room temperature of about 25° C., growing well at temperatures of 15° to 30° C.

Ascomatibus raris, in cortice et ligno initio subimmersis, dein erumpentibus, singulis vel gregariis, disco membrana pulveracea griseoalba tecto, ostiolo punctiforme, 0.7–1.0 mm. diam.; ascis cylindraceis, brevipedicellatis, 170–200 × 8–12 μ, crasse tunicatis; paraphysibus filiformibus, simplicibus, pluriseptatis 1.3–1.7 μ diam; sporidiis filiformibus, parallelis ascorum longitudine, 1 μ diam. pluriseptatis, mox in articulos 2–3 μ longos secedentibus.

Pycnidis communibus, in cortice et ligno subimmersis, erumpentibus, membrana pulveracea griseo-alba tectis, singulis vel gregariis, ostiolo carente; pycnoconidiis histogenicis, pycnidium implentibus, cylindraceis, rectis curvatives, 2–5 × 1 μ.

[7] The authors wish to acknowledge the assistance of Edith K. Cash, Assistant Mycologist, United States Department of Agriculture, in attempts to identify this fungus and in writing the Latin descriptions.

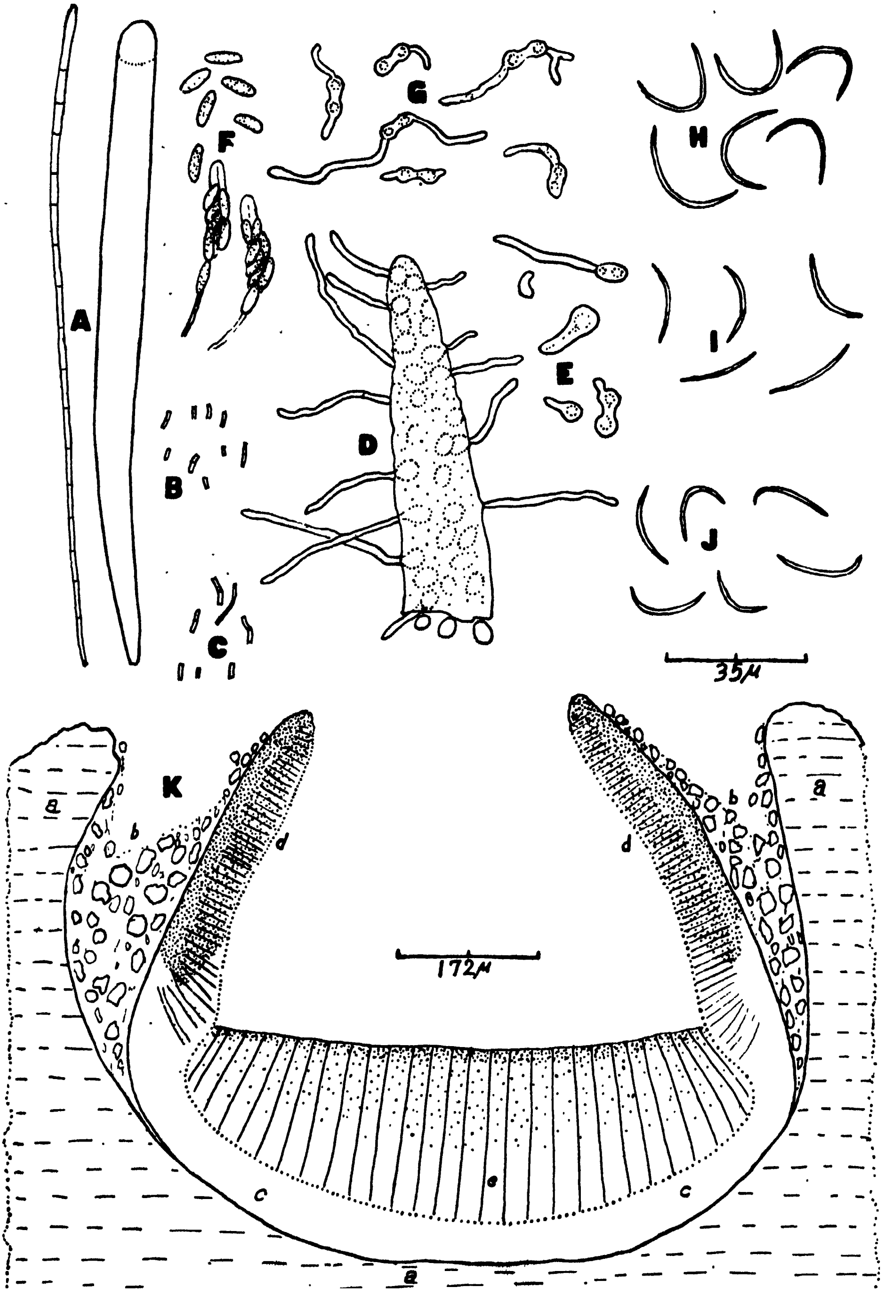

FIG. 5. A to E and K. *Schizoxylon microsporum*, n. sp. A. Paraphysis and ascus. B. Segments of ascospores. C. Pycnospores. D. Tip of ascus after 20 hours on corn-meal agar showing germination of ascospores. E. Segments of ascospores showing early stages of germination. F to H. *Eutypella parasitica*, n. sp. from maple canker. F. Asci and ascospores. H. Conidia from 6-week-old culture. I. Conidia from ascospore culture of *Eutypella angulosa*. J. Conidia from ascospore culture of *Eutypa heteracantha*. K. Sketch of a section of apothecium of *Schizoxylon microsporum*: a, wood; b, white crystalline material; c, hypothecial layer; d, black upper covering; e, ascus layer.

On cankered *Acer saccharum* Marshall and *A. rubrum* Linn. in Minnesota, Wisconsin, and Michigan. Specimen number 71569 type, pycnidial specimen Nos. 71450, 71451, and 71571.

SUMMARY

Two cankers, *Eutypella* and *Schizoxylon,* are reported on sugar and red maples and described for the first time. Eutypella canker, which has dense white to light buff mycelial fans under the bark at the margin, has *Eutypella parasitica,* n. sp. commonly fruiting on it. The other has associated with it the pycnidial stage of *Schizoxylon microsporum,* n. sp. The ascus stage was found on one canker and cultures from ascospores were the same as cultures from pycnospores.

The Eutypella canker is common in the Lake States and has been collected in New York, Vermont, and New Hampshire. Schizoxylon canker occurs only occasionally throughout the Lake States. Both appear to cause conspicuous killing of the bark and distortion of the trunk.

Both fungi have been repeatedly isolated from margins of active cankers and from discolored wood back of killed areas, and such isolates appear to be identical with cultures from ascospores. A few inoculations were made on sugar maple, and the fungi were reisolated in a few cases but insufficient time had elapsed to get typical canker formation.

DIVISION OF FOREST PATHOLOGY, BUREAU OF
PLANT INDUSTRY, AND CIVILIAN CONSERVATION CORPS,
UNITED STATES DEPARTMENT OF AGRICULTURE.

GRAVITY GRADING, A METHOD FOR REDUCING SEED-BORNE DISEASE IN COTTON

K. STARR CHESTER

(Accepted for publication July 16, 1938)

Cottonseed is commonly infected to a serious extent with pathogenic organisms, particularly *Glomerella gossypii* Edg., *Bacterium malvacearum* E. F. S., *Fusarium moniliforme* Shel. and other species of *Fusarium.* These organisms are present in the lint and on the surface of the seed, as well as within the seed coat. They are so destructive in the stages of germination and emergence that it is not uncommon to use a seeding rate 10 or more times in excess of the expected stand. Of the various suggested methods for controlling cottonseed pathogens, 2 are outstanding in their beneficial effects, *i.e.,* delinting with concentrated sulphuric acid, and dusting with organic mercury preparations such as 2% Ceresan. While these 2 means of surface disinfection are advantageous, they do not completely solve the problem of cottonseed treatment, since neither will prevent the ultimate activity of pathogens within the seed coat. The planting of a single internally-infected seed in a hill is a hazard to the remaining healthy seed in the same hill.

Even acid- or Ceresan-treated seed lots commonly contain 10 per cent or more of internally infected seed, and these are capable of infecting many of the remaining healthy seed before the stand is established. The purpose of this paper is to report a practical method that to a large extent eliminates the internally infected seed.

Briefly, the method consists of acid-delinting followed by covering the seed with water and rejecting the fraction that floats. The acid-delinting is carried out according to standard methods using 9 lb. ($\frac{1}{2}$ gal.) of 66° Baumé sulphuric acid per bushel of fuzzy seed. Half of this amount of acid is sufficient if gin-delinted seed are used. The delinted seed are washed in the usual manner. When washing is complete, the seed are allowed to settle in the water, and the fraction which rises to the surface is decanted or removed with a screen. The two fractions, hereinafter referred to as "light" and "heavy," are dried and bagged separately. Ordinarily, about 40 per cent of the whole seed, by weight, fall into the light fraction (average of tests of 19 seed lots). The heavy fraction is used for planting; the light fraction may be sold to cottonseed processors at a satisfactory price.

The superior quality of the heavy fraction has been seen in numerous laboratory, greenhouse, and field tests. The results of these tests are here briefly summarized, and details of the tests are reserved for a later publication.

Laboratory germination tests comprised 180 germinations of 26 lots of cottonseed. The "rag doll" method was followed using 100 seed to a "doll," and only "clean germination" was scored, *i.e.*, seedlings with obvious infection such as to preclude survival were classed with ungerminated seed. Ordinarily, a test consisted of the germinations of 4 samples from the same source: (a) fuzzy seed, (b) the same after delinting but without further fractionation, (c) the light fraction of (b), and (d) the heavy fraction of (b). Averaging all experiments, the heavy fraction (d) proved to be considerably superior to all other samples. The heavy seed produced 2.2 times as many healthy seedlings as the light seed, and 1.24 times as many healthy seedlings as the whole delinted fraction (b). In many cases the heavy seed produced 99 or 100 healthy seedlings from 100 seed, while the whole delinted fraction produced about 80 healthy seedlings and the light fraction about 50.

The percentage of germination, however, gives only a partial measure of the superiority of the heavy seed, since the seedlings from heavy seed proved more vigorous than the seedlings from the other fractions.

Whole delinted seed usually produced a higher percentage of healthy seedlings than fuzzy seed of the same source. However, the more striking gain in clean germination after fractionating the delinted seed indicates that internal infections are more important in cottonseed pathology than the surface infestations, which can be controlled by chemical methods.

Greenhouse germination tests in sterilized and in unsterilized soil gave essentially the same results as the laboratory germination tests.

In the field, the method was tested in 3 plantings in the spring of 1938. In each planting the seed were commercial lots from local sources, four randomized replications were used, and the heavy seed were tested in comparison with light seed, whole delinted seed, and fuzzy seed, all originally from the same sack. Each of the seed lots was hand-planted in four 50 ft. rows in hills of exactly 5 seed each, at 10″ intervals. The weather was well adapted to a test of the method under both favorable and adverse conditions.

Planting No. 1 (April 24) was followed by cool wet weather. The same planting included 16 fuzzy seed lots, some treated with "Ceresan" (Cotton Disease Council, 1938 cooperative seed treatment tests). Of the 80 rows planted, only 4 produced useable stands; these 4 were the 4 replications of heavy delinted seed. The heavy seed produced 4.5 times as many seedlings per row as the average for the 19 remaining seed lots.

Planting No. 2 (May 13) was followed by warm rains. This planting was an exact repetition of planting No. 1 except that the randomization was independent. Most of the seed lots gave stands that were usable, although not outstanding. Averaging the four replications, the heavy seed fraction produced twice as many healthy seedlings as the whole delinted fraction and 3 times as many as the light fraction.

Planting No. 3 (May 21) was followed by ideal conditions for cottonseed germination. The planting was also as in the former two, but independently randomized. The stands of the better seed were excellent. The four replications gave uniform results with relatively small variation. The averages of the replications showed a healthy emergence of the heavy fraction, which was 1.5 times as great as that of the whole delinted seed and 2.4 times as great as that of the light fraction.

The field experiments afford further evidence that the heavy fraction of delinted seed produces a significantly higher percentage of healthy seedlings than whole delinted seed. This superiority of the heavy fraction was maintained under poor, average, and good conditions for germination. The agreement of field, greenhouse, and laboratory tests, and the similarity of the results obtained with numerous seed lots, indicate that the method of gravity-grading appears to have a general application in cottonseed improvement. As yet we are unable to state the effect of gravity-grading on the yields or quality of cotton. However, it is known that the yields from even stands usually exceed those from broken stands, and an increased yield would be anticipated from any practice that tends to eliminate skips in the row.

The use of gravity-grading in farm practice appears to be practicable. Acid delinting has not become an established practice in many parts of the cotton belt, largely because of the lack of suitable equipment. At present the grower is limited to two alternatives, home delinting in tubs, or the installation of the seed-delinting machinery devised in Arizona.[1] The home

[1] BROWN, J. G., and F. GIBSON. A machine for treating cotton seed with sulphuric acid. Ariz. Agr. Exper. Sta. Bull. 105. 1925.

method is unpopular because the average cotton grower is unable to handle concentrated sulphuric acid with safety and efficiency. The Arizona machinery is expensive to construct and operate, and in some cotton areas its cost may be prohibitive. It is believed that delinting machinery can be constructed that will be reasonably inexpensive and that can be made generally available on a community basis, at a low cost. Provided the expense of delinting equipment is not excessive, the cost of delinting and fractionating is more than offset by the following facts:

1. Heavy delinted seed can be sown at ⅓ the seeding rate for fuzzy seed, and still produce good stands under optimum conditions.
2. The light fraction can be sold for a satisfactory price as is indicated by such quotations from cottonseed processors as the following:

   "On a clean seed basis, our laboratory finds that the total oil is 323 lb. per ton and the available oil 258 lb., and on the basis of 5% manufacturing loss these seed will produce 1,028 lb. of 43 per cent meal to the ton. Such seed would be desirable for crushing purposes, and we believe that any mill would buy them in any quantity on the basis of the market value of prime seed." (Southwestern Cotton Oil Co., Oklahoma City, Okla.)

   The light fraction may also be suggested for trial as a superior livestock feed by virtue of its low percentage of fiber. Most or all of the price received for the light fraction is profit, since the heavy fraction can be used to plant an acreage equal or nearly equal to that that would otherwise be planted with whole seed.
3. The heavy seed are not only relatively disease-free but, in addition, they represent the seed with the largest amounts of reserve food. This implies greater vigor throughout the early period of seedling development.

So far as is known to the writer, the only previous reports on the weight of cottonseed in relation to successful culture are those of Kottur, Staten, and Taubenhaus and Burkett. Kottur in India applied a brime flotation technique to fuzzy seed and reported better germination with the heavy fraction.[2] He stated that the method was not applicable to American seed. We have attempted this method, with additional variations, and in no case have been able to obtain a satisfactory fractionation of American seed. Staten in 1933[3] weighed individual seed and showed that the heavier seed produce more vigorous plants. These gave larger yields than the plants from small seed during the first year after selection. Yields the second year were reported to be less in favor of the heavy seed. No method was proposed for the practical application of this finding. It should be pointed out that our method differs from that of Staten in that it depends

[2] KOTTUR, G. L. A simple method of selecting heavy seeds in cotton. Poona Agr. Col. Mag. 8: 203–210. 1917. (Abstracted in Experiment Station Record 40: 237–238. 1919.)

[3] STATEN, H. W. The comparative effects of light and heavy seed in cotton. Proc. Assoc. So. Agr. Workers 34: 84. 1933.

upon the specific gravity of the seed and not on their absolute weight. Measurements by G. Tennyson[4] indicate that there is little or no correlation between absolute weight and specific gravity of cottonseed after eliminating a small fraction of immature seeds and empty hulls. Internally infected seed are well distributed through all of the absolute weight classes.

In the Texas Agricultural Experiment Station Report for 1936[5] Taubenhaus and Burkett very briefly mention grading delinted cottonseed by the use of specific gravity. Irrespective of subsequent treatments with fungicides, the seed that were heavier than water were superior to ungraded control samples as regards germination percentage, rapidity of emergence, and final stand produced.

The work herein reported is being continued with particular reference to the morphological and pathological basis of low specific gravity in the seed and to the construction and use of equipment for delinting and gravity-grading on a practical scale.

SUMMARY

Cottonseed were delinted with sulphuric acid and then suspended in water and divided into the fractions that sank and floated, respectively. Laboratory, greenhouse, and field experiments on germination and emergence indicate that nearly all internally infected and weak seed are removed by this method. Healthy germination and emergence of the heavy seed was over twice as great as that of the light seed and half again as great as that of ungraded delinted seed. Practical application of the method appears feasible.

INCUBATION PERIOD OF PEA VIRUS 1 IN THE APHID MACROSIPHUM SOLANIFOLII

H. T. OSBORN

(Accepted for publication June 24, 1938)

In a previous paper[1] it was shown that both the pea aphid, *Macrosiphum pisi* Kaltenbach, and the potato aphid, *M. solanifolii* Ashmead (*M. gei* Koch), are vectors of pea virus 1. Transmission of pea virus 1 by the pea aphid is of considerable interest, because it is necessary for the virus to undergo an incubation period of about 12 hours before it can be transmitted by the insect and also because the aphid retains the ability to transmit the disease for long periods of time. When the paper was published, little was known about transmission of pea virus 1 by the potato aphid. Recent experiments have shown, however, that the virus undergoes a similar

[4] Communicated by Miss Tennyson and cited with her permission.

[5] TAUBENHAUS, J. J., and A. L. BURKETT. Control of damping-off of cotton seedlings. Texas Agr. Exp. Sta. Rep. 49: 105. 1936; and Germination and yields from acid-delinted cotton seed. *Ibid.* 49: 109. 1936.

[1] Osborn, H. T. Incubation period of pea mosaic in the aphid, *Macrosiphum pisi*. Phytopath. 25: 160–177. 1935.

period of incubation in the potato aphid and that it is retained in this aphid for a considerable period of time. Further tests were made also with the bean aphid, *Aphis rumicis* L. It is the purpose of this paper to present the data from these experiments.

MATERIALS AND METHODS

The virus used in most of the experiments was the same strain of pea virus 1 that was employed in studies on transmission by the pea aphid. It had been maintained in a greenhouse for a period of 6 years by successive transfers to fresh plants by means of the pea or potato aphids. In a few experiments another strain of pea virus 1 was used. This was obtained originally from pea aphids collected on pea plants growing near Princeton, New Jersey.

Colonies of the pink form of the potato aphid[2] were maintained in screened cages in a greenhouse on tomato, *Lycopersicon esculentum* Mill., Hubbard squash, *Cucurbita maxima* Duchesne, or potato, *Solanum tuberosum* L. The most vigorous colonies were produced on potato. Colonies of the bean aphid[2] were maintained on *Vicia faba* L

In the experiments to be described, aphids from colonies on healthy plants were transferred by means of a camel-hair brush to diseased *Vicia faba* plants, where they were allowed to remain for periods of from 3 hours to 2 days. They were then transferred to a succession of healthy *V. faba* plants which were grown in 2½-inch pots. Immediately after exposure, the healthy plants were fumigated with Nicofume and returned to a greenhouse where they were grown beside noninoculated control plants.

INCUBATION PERIOD IN THE POTATO APHID

To determine whether or not an incubation period of pea virus 1 in the potato aphid is necessary for transmission, experiments similar to those previously reported for the pea aphid were undertaken. Although potato aphids ordinarily do not colonize on *Vicia faba* or peas, they feed readily when transferred to these hosts and, in previous experiments, had proved efficient carriers of the virus when fed for a day on diseased *V. faba* plants before transfer to healthy plants. They were found to be more easily injured than pea aphids. When transferred frequently at short intervals, the size of the colonies rapidly diminished. For this reason the results of several experiments were unsatisfactory because only a few of the plants exposed became diseased.

In one experiment, however, 5 colonies, each consisting of 75 potato aphids, were fed for 3 hours on diseased plants. They were then transferred to a succession of healthy plants at intervals of 3 hours until 9 sets of plants had been exposed. The 10th period of exposure continued for 17 hours and the final period for 3 days. The temperature of the green-

[2] Identification of aphids from these colonies was verified by Dr. P. W. Mason, Division of Insect Identification, U. S. Bureau of Entomology and Plant Quarantine.

house varied from 74° to 84° F. during this period. At the 10th transfer there were only 13 aphids in the 5 colonies. All were dead at the end of the final period. The results of the experiment are shown in table 1. Two

TABLE 1.—*Infections obtained with 5 colonies of the potato aphid when fed for 3 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Length of successive exposure periods (hours)	Approximate number of aphids on each plant	Succession of healthy plants				
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
3	75	–	–	–	–	–
3		–[b]	–	–	–	–
3		–	–	–	–	–
3		–	–	–	–	–
3		–	–	–	–	–
3		–	–	–	+	+
3		+[a]	–	–	–	–
3		–	–	–	–	–
3	13	–	–	–	–	+
17		–	–	–	+	+
72		–	–	–	–	+

[a] + = plant became diseased.
[b] – = plant remained healthy.

colonies became infective at the 5th period of exposure and one colony at the 6th, thus demonstrating an incubation period of not less than 12 nor more than 18 hours in 2 of the colonies and of not less than 15 nor more than 21 hours in a 3rd colony. Failure to infect consistently in subsequent periods of exposure is believed to have been due mainly to loss of infective individuals in the colony. Two colonies apparently failed to acquire the virus during a feeding period of 3 hours on diseased plants.

In a final experiment, potato aphids from a vigorous colony that had been reared on potato were used as vectors. Large-size nymphs and adults were fed for 4 hours on 8 *Vicia faba* plants showing severe symptoms of disease. At the end of this period the aphids were removed and transferred to a succession of fresh plants. Six sets of fresh plants were exposed at intervals of 4 hours. The aphids were then fed for 20 hours on potato. On the second day a fresh set of 8 *V. faba* was exposed for 4 hours. The aphids were then again fed on potato for 20 hours, and on the third day a final set of 8 fresh *V. faba* plants was exposed. A temperature of 74° to 78° F. was maintained throughout the experiment. Approximately 100 aphids were placed on each of the healthy plants during the first period of exposure. By the 4th period the colonies had been reduced to an average of 80 aphids, and by the end of the 6th period to an average of 55 aphids. At the end of the 8th period there were still approximately 50 aphids in each colony.

The infections obtained in this experiment are shown in table 2. No infection occurred in any of the plants exposed during the first 3 periods.

TABLE 2.—*Infections obtained with potato aphids when fed for 4 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Length of successive exposure periods (hours)	Approximate number of aphids on each plant	Number of plants exposed	Number of plants that became diseased
4	100	8 *V. faba*	0
4		"	0
4		"	0
4	80	"	2
4		"	3
4	55	"	3
20		Insusceptible potato	0
4	50	8 *V. faba*	6
20		Insusceptible potato	0
4		8 *V. faba*	5

Two plants were infected at the 4th period, 3 at the 5th period, and 3 at the 6th period. Six of 8 plants were infected when exposed for 4 hours on the 2nd day and 5 of 8 when exposed for 4 hours on the 3rd day.

The experiment demonstrated a minimum incubation period of not less than 12 nor more than 20 hours for some of the aphids in the colony. It also demonstrated retention of the virus by the colony for a period of at least 3 days.

RETENTION OF VIRUS BY APHIDS WHEN FED ON INSUSCEPTIBLE PLANTS

To determine whether the virus is retained by the aphids for periods longer than 3 days, they were fed on diseased *Vicia faba* plants for 1 day or more, transferred to tomato for a suitable period of time, and then returned to healthy *V. faba* plants. Tomato plants were used in these experiments, because they serve as satisfactory food plants for the potato aphid and at the same time are immune from infection by pea virus 1.

In one experiment approximately 500 large-sized nymphs and adults of the potato aphid that had fed on diseased *Vicia faba* plants for 24 hours were transferred to 4 small tomato plants. Transfers to fresh tomato plants were made at intervals of several days in order to remove newborn nymphs. After 14 days, the colony had dwindled to 100 individuals. These were tested for retention of the virus by transfer to 5 fresh *V. faba* plants for a period of 2 days. They were then fed for 4 days on tomato plants before being tested again by placing them on healthy *V. faba* plants for 2 days. Only 32 individuals were alive at the beginning of the final test. Of the 5 plants exposed to 100 potato aphids that had fed on insusceptible tomato plants for 14 days, all became diseased. Of the 5 *V. faba* plants exposed to 32 aphids in the final test, 4 became diseased. The infections obtained in this experiment demonstrated that the potato aphid retained the virus during a feeding period of 14 days on insusceptible tomato plants and during a total feeding period of 20 days.

In another experiment, potato aphids were fed for 2 days on *Vicia faba* plants diseased with the strain of pea virus 1 obtained near Princeton,

New Jersey. On removal from the diseased plant, 75 of the aphids were transferred to 5 healthy *V. faba* plants, while 350 were placed on tomato plants. The aphids on tomato plants were transferred at intervals of several days in order to destroy newborn nymphs. After a feeding period of 14 days on tomato, 75 aphids were removed and transferred to 5 small *V. faba* plants. After 21 days, only 27 aphids remained on the tomato plants and these appeared inactive and weak. The 27 aphids were removed from tomato at this time and placed on 5 small *V. faba* plants. Of the 5 *V. faba* plants exposed to 75 potato aphids immediately after removal from diseased plants, all became infected. Of the 5 plants exposed to 75 aphids that had fed on tomato plants for a period of 14 days, all became infected, while of the 5 *V. faba* plants exposed to 27 aphids that had fed on tomato for 21 days, one became infected. This experiment demonstrates that potato aphids, having once acquired the virus, may retain it during a continuous feeding period of 21 days on insusceptible tomato plants.

EXPERIMENTS WITH THE BEAN APHID

In previous experiments[3] the bean aphid failed to transmit pea virus 1, but did prove to be an efficient vector of pea virus 2.[4] Since the aphid feeds readily and multiplies rapidly on *Vicia faba*, it seemed desirable to conduct further tests to determine whether or not it might occasionally transmit pea virus 1.

For this purpose bean aphids were colonized on diseased *Vicia faba* plants for 4 or 5 days. Heavily infested leaves were then cut off and placed beside small healthy plants so that the aphids migrated naturally from the diseased to the healthy plants. In one series 50 small *V. faba* plants were exposed to aphids that had fed on *V. faba* plants diseased with the strain of virus from New York. In another series 100 small plants were exposed to a strain of the virus secured near Princeton, New Jersey. Of the 150 small plants exposed, not one developed symptoms of pea virus 1. This result confirms previous experiments and demonstrates conclusively the inability of the bean aphid to transmit this virus.

DISCUSSION

In nature, the pea aphid undoubtedly is the principal vector of pea virus 1, since it occurs in abundance on legumes susceptible to the virus. The potato aphid, also capable of carrying the virus, commonly occurs on insusceptible solanaceous hosts and for this reason is probably not an important vector in the field. It seemed of interest, nevertheless, to determine the mode of transmission of pea virus 1 by the potato aphid, as well as by the pea aphid, because an incubation period has been shown for only one other virus transmitted by aphids. Potato-leaf-roll virus undergoes

[3] *Loc. cit.*
[4] Osborn, H. T. Studies on the transmission of pea virus 2 by aphids. Phytopath. 27: 589–603. 1937.

an incubation period in the green peach aphid, *Myzus persicae* Sulzer.[5,6] The results of the experiments show definitely that transmission of pea virus 1 by the potato aphid involves an incubation period and that the virus is retained by the aphids in a manner similar to that previously shown for the pea aphid.

SUMMARY

By exposing successions of healthy *Vicia faba* plants to colonies that had fed for short periods on diseased plants, it was shown that pea virus 1 undergoes an incubation period in the potato aphid, *Macrosiphum solanifolii.* The minimum incubation period demonstrated was not less than 12 nor more than 18 hours.

Potato aphids that acquired the virus were shown to retain it for as long as 21 days when fed continuously on insusceptible tomato plants.

The bean aphid, *Aphis rumicis,* failed to transmit the virus.

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PHYTOPHTHORA ROT OF ASPARAGUS IN CALIFORNIA

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(Accepted for publication July 15, 1938)

In the middle of March the attention of the writers was called to a soft rot in asparagus shipped from Valdez, California, to Chicago and New York.

The examination of asparagus spears that were delivered to the packing house from nearby fields showed definite lesions on the stem. These lesions were in the form of spots of different sizes, water-soaked and soft (Fig. 1). This type of asparagus spear was usually discarded by workers before bundling the asparagus preliminary to packing and precooling. Loose asparagus, as well as bundled, was subjected to laboratory study. Spears with definite lesions were cultured. The cultured spears yielded pure cultures of *Phytophthora* sp. that, upon inoculation into healthy asparagus, produced infection similar to that observed on cultured spears. Upon reculturing, a pure culture of *Phytophthora* sp. was isolated in all particulars similar to the one introduced. The *Phytophthora* sp. was isolated from asparagus from the above-mentioned localities by C. M. Tompkins of this Division in March, 1936, and recently reported[1] to be found in the New York markets on asparagus from this area. The *Phytophthora* sp. causing this

[5] Elze, D. L. De verspreiding van virusziekten van de aardappel (*Solanum tuberosum* L.) door insekten. Meded. Inst. Phytopath. Lab. Mycol. Aardappel-onderzoek, Wageningen 32: 1927.

[6] Smith, K. M. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. Am. Appl. Biol. 18: 141–157. 1931.

[1] Wiant, James S., and C. O. Bratley. Diseases of fruit and vegetables on the New York market during January, February, March, and April, 1938. Plant Dis. Reptr. 22 (11): 190–193. 1938.

disease in asparagus is being studied by the junior writer and an account of it will be published soon.

On field material an abundance of oospores and sporangia was observed and discharge of zoospores occurred when specimens were placed in water. When the tips of asparagus are affected by the *Phytophthora* sp. a softening of this part of the plant is developed, which, being accompanied by saprophytic bacteria, produces an extremely vile odor. The bacteria accompanying the Phytophthora rot were not found capable of attacking the healthy tips when inoculations were made with pure cultures.

To ascertain the degree of infection in asparagus that was bundled and ready for precooling, bundles of asparagus spears were brought to the laboratory and stored at 41° F. for a period of 5 days, after which a count of

FIG. 1. Stalk rot of asparagus caused by *Phytophthora* sp. Naturally infected stalk of French Argentile variety on top, healthy stalk at bottom.

diseased spears was taken. A representative number of diseased spears was cultured to be sure the symptoms observed were produced by *Phytophthora* sp. The tests showed that the infection ran from 6 to 18.5 per cent.

Similar conditions existed in a packing house at Antioch, California. Through the courtesy of Mr. Lewis Lawyer several bundles of asparagus were obtained and, upon examination, were found to contain from 20 to 30 per cent infected spears after 5 days' incubation at 41° F. These tests clearly indicated, (a) that the Phytophthora rot was present in the field and (b) that in spite of inspection prior to the bundling, infected spears escaped the inspector's notice. Furthermore, it was important to establish if the *Phytophthora* sp. was present in the water used in the precooling operation. The water samples collected after a day's run of the precooler, when cultured, yielded numerous colonies of the *Phytophthora*. It was not shown, however, that the practice of precooling in water had any pronounced influence on the ultimate amount of disease.

This situation raised the pertinent question of the possibility of water disinfection with the simultaneous disinfection of the asparagus spears that might have either the early stages of the disease or the zoospores lodged on them. With this idea in mind a number of disinfectants was tried out under laboratory conditions. The chemicals were tested on the fungus and also on asparagus spears. To test toxicity of the selected chemicals the fungus was grown on a potato-dextrose-agar plate and then cut into small discs by means of a sterilized cork borer. These bits of fungus were dropped into the test solutions for various intervals of time and, after washing in sterile distilled water, were placed into potato-dextrose-agar plates with subsequent incubation at room temperature. These tests showed that *Phytophthora* sp. was killed as follows: by 2 per cent of Labarraques solution (2.6 per cent sodium hypochlorite) in 15 minutes; by ¼ per cent of commercial ammonium hydroxide (28 per cent strength) in 10 minutes; by ¼ per cent of sodium peroxide in 5 minutes; by ½ per cent sodium peroxide in ½ minute; by 0.05 per cent sodium peroxide in 5 minutes. The organism was killed in one minute by water at 46° C. The test showed no injury to tender asparagus spears by the above chemicals and hot water for the duration of the experiments. Treatments of asparagus with field infections, as well as asparagus artificially inoculated in the laboratory by dipping into a water suspension of the zoospores, showed a considerable efficacy in checking the trouble under the experimental conditions.

The disease appeared to be related to a period of heavy and prolonged rainfall and perhaps to the rather common practice of flooding certain areas to produce earlier growth.

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PHYTOPATHOLOGICAL NOTES

Nematode Infestation of Olive Roots.—For the past several years it has become increasingly evident that fig trees in California are seriously affected by a species of nematode[1] different from that causing the well-known root-knot or bead-like swellings of the fibrous roots. The former infests the bark of the larger roots, resulting in necrotic areas and often in complete destruction of the affected root, especially of the taproot.

Recently, the writers had occasion to observe the roots of 10-year-old olive trees that were being removed. These alternate trees in fertilizer plots at this Station were apparently normal and healthy as to the tops. The roots of many, however, showed an abnormal condition manifested by longitudinal cracks in the bark, some being black and necrotic, others whitish and callused. Specimens were sent to Dr. G. Steiner, United States Department of Agriculture, Washington, D. C., who reported that the "roots

[1] Gerald Thorne. Some plant parasitic nemas, with descriptions of three new species. Jour. Agr. Res. [U. S.] 49: 755–763. 1934.

are infested with the same nematode species previously reported from the fig tree roots which you sent in. In our conception, the species is *Pratylenchus musicola* and not *Pratylenchus pratensis* as reported by Thorne," the differentiating characters of the two species having been very vague in the past.

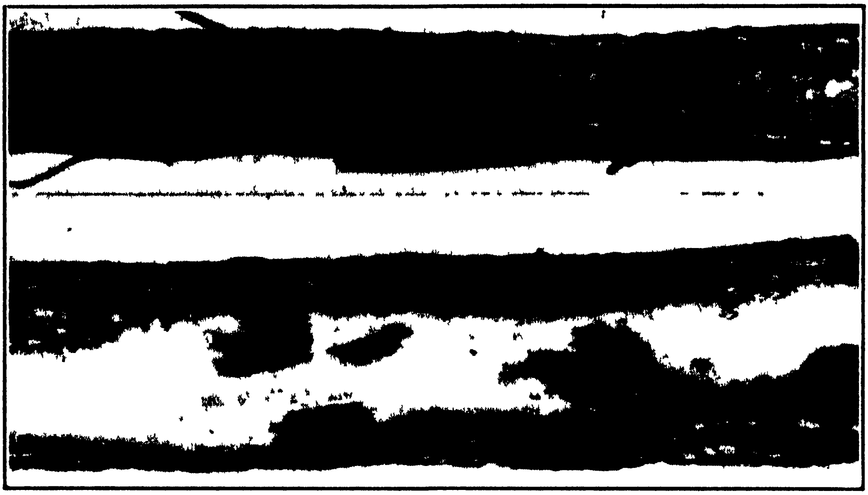

FIG. 1. Roots of olive trees at the Citrus Experiment Station, Riverside, California, showing lesions in which the nematode, *Pratylenchus musicola*, occurs abundantly.

This nematode apparently attacks only the bark portion of the olive root and does not kill the cambium nor cause a rotting of the larger roots, as it does in figure 1. Until further observations are made on its prevalence in olive districts, we are unable to state what effect infested roots may finally have upon the nutrition and productiveness of this new host plant.—IRA J. CONDIT AND W. T. HORNE, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

A Phloem Necrosis of Elm.—For several years an epidemic dying of American elms has occurred in the central and lower Ohio River watershed. In Ohio it was first reported at Ironton in 1918 and at Dayton in 1927. Since then a similar condition has obtained in many localities throughout the region. This dying was probably prevalent in southwestern Indiana and northern Kentucky before it was discovered in Ohio. It is impossible at present to determine whether all these epidemics have been due to the same cause.

In Chillicothe, Ohio, an epidemic of this type began in 1935. In the spring of 1936 an investigation was begun there and elsewhere. A thousand trees, 50 per cent of the city's elms, died in 1936 and 1937. Affected trees died in from 3 to 36 months after the first apparent symptoms. No recovery was observed. The disease is extremely virulent and has spread rapidly in Ohio on the American elm.

Symptoms are first noticeable in the extreme top at outer tips of branches. The foliage becomes thin, the leaves droop, because of downward curvature of the petioles. The leaf blade curls upward at margin, producing a trough-like effect that makes the leaves appear narrow and greyish-green and entire crown of tree thin. Such leaves are often stiff and brittle. Later, foliage becomes yellowish-green and finally yellow, followed by defoliation. These symptoms usually occur throughout the entire crown; are not confined to one or several branches, as is frequent with common wilts; there is no vascular discoloration.

In fairly advanced stages of the disease the roots die, the small fibrous ones first. Typical discoloration, confined to phloem and cambium, precedes death of larger roots and may be found frequently extending into trunk and branches. In large trees this discoloration usually is found in large roots and at base of trunk, just before death. The cambium first becomes light yellow or golden. The phloem becomes yellow in region adjacent to cambium, then brown, with small, scattered, black flecks. Soon thereafter, phloem becomes dark brown and necrotic. An odor resembling wintergreen characterizes moderately discolored phloem.

Over 4,000 attempted plate isolations, averaging 10 plantings each, have been made from the roots, trunks, and branches of over 400 diseased trees. No organism has been secured consistently; none appears associated with the disease. Over a 2-year period inoculations with the organisms obtained have failed to indicate their pathogenicity.

Histological study of diseased tissue has shown no organism consistently present. In almost all cases the organisms found were confined to the dead roots.

Direct insertion of diseased tissues into 72 healthy elms has not resulted in transmission.

Healthy elms with injured roots planted in soil secured from around diseased trees in the field remained healthy. Planting healthy elms with artificially injured roots in a compost consisting of 50 per cent diseased roots and 50 per cent soil by weight resulted in no transmission.

In July, 1937, 21 healthy American elms were grafted with patches of bark from diseased trees, the patch showing typical phloem discoloration. Five nongrafted trees were left at random as checks. In August, 1938, the disease was apparent in 14 grafted trees, all showing complete symptom expression. The nongrafted check trees in this block and approximately 400 other elms in the nursery remained healthy.

In January, 1938, 20 healthy trees were grafted with branch scions from diseased trees, and the roots of 26 healthy elms were grafted with diseased root scions. Four healthy trees were grafted with healthy branch scions and 4 with healthy root scions as checks. These trees were planted in the greenhouse. Of the branch-grafted trees, union between scion and stock occurred on the 4 check trees and on 15 of those grafted with diseased scions. Up to September 12, 1938, all checks and trees unsuccessfully grafted remained

healthy. Of the remaining 15 trees, transmission and complete symptom expression were obtained on 13.

Up to September 12, 1938, transmission and complete symptom expression were obtained on 5 trees grafted with diseased root scions. All check trees remained healthy. Whether union between diseased root scion and healthy stock was secured on the remaining trees can not be determined until the trees are dug after they are dormant.

From the experiments undertaken, it appears that this disease is of a virus nature and that it is systemic. How it is transmitted under natural field conditions is yet unknown.—ROGER U. SWINGLE, Division of Forest Pathology, U. S. Department of Agriculture, in cooperation with the Ohio Agricultural Experiment Station, Wooster, Ohio.

Preliminary Tests to Determine Effect of Arsenite Sprays on Sporodochia of Sclerotinia laxa and on Control of Brown Rot in Blossoms of Almond and Apricot.—By spraying apple leaves in the autumn with calcium and zinc arsenite in combination with copper sulphate and lime, Keitt and Palmiter[1] prevented the development of ascospores by *Venturia inaequalis* (Cke.) Wint. They were able also to suppress the production of primary inoculum by other fungi.

The present writers wish to report the results obtained by spraying almond and apricot trees with arsenite sprays for control of the brown rot caused by *Sclerotinia laxa.* The sporodochia are the major if not the only source of primary inoculum for blossom infection in these crops. They are produced abundantly during spring on mummied fruit and on twigs blighted the previous year.[2]

In 1936, apricot trees sprayed with a sodium arsenite solution at the time blossoms were emerging from the bud (after sporodochia had appeared), developed markedly less blossom infection than nonsprayed trees. In 1937 apricot trees again were sprayed in the same stage with various concentrations of sodium arsenite and with monocalcium arsenite (4 pounds to 100 gallons). As trees in this orchard failed to develop blossoms, no observations on incidence of the disease could be made.

In 1938 monocalcium arsenite, zinc arsenite, and sodium arsenite were applied to almond and apricot trees. As the purposes of these tests were to determine the effect of the sprays on the development of sporodochia, on germinability of sporodochium conidia, and on subsequent development of blossom infection, the applications were made both before and after sporodochia appeared in the trees.

Sodium arsenite (1.2 pounds to 100 gallons of water), applied to almond trees before sporodochia appeared, had no apparent effect on their development, and little, if any, effect on development of blossom infection. This material (1–100), however, applied to apricot trees after sporodochia ap-

[1] Keitt, G. W. and D. H. Palmiter. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. Jour. Agr. Res. 55: 397–437. 1937.

[2] According to unpublished data by Wm. B. Hewitt and L. D. Leach.

peared, markedly reduced germinability of the conidia and prevented blossom infection to a considerable extent.

Monocalcium arsenite (4–100) plus 4 per cent of a dormant type petroleum oil emulsion, applied to almond trees before sporodochia appeared, suppressed sporodochium development 96 per cent and reduced the incidence of blossom infection 80 per cent. When applied after sporodochia had appeared, this material reduced germinability of conidia 97 per cent and the incidence of blossom infection 71 per cent.

Zinc arsenite (4–100) with or without 4 per cent oil emulsion was not so effective in preventing sporodochium development. When applied after these structures appeared, however, zinc arsenite (3–100) plus 2 per cent oil emulsion, reduced germinability of conidia 90 per cent and the incidence of blossom infection 83 per cent.

Although too meagre for final conclusion, these results are sufficiently promising to warrant further tests. Much is yet to be done in determining the effect of the materials on the tree.—E. E. WILSON and E. F. SERR, Division of Plant Pathology and Agricultural Extension Service, respectively, University of California, Berkeley, California.

Inoculation of Conifers with the Cypress Coryneum.—A canker caused by *Coryneum cardinale* has been reported by Wagener[1, 2] as attacking and eventually killing trees of Monterey cypress, *Cupressus macrocarpa*.[3] This disease also can attack other species of *Cupressus*[2] but to a lesser degree. It has reduced a planting of *C. macrocarpa* on the campus of the Citrus Experiment Station to a few trees and these are now severely attacked. *Cupressus sempervirens* and *C. glauca* (*C. arizonica, var. bonita*) growing near have apparently escaped.

Inoculations were made through wounds made in the bark of small trees by means of a 4 mm. cork-hole borer. Two methods of inoculation were used: (1) Placing the mycelium of a pure culture in the wound, and tying over it some moist absorbent cotton. Then the whole is wrapped with paraffin paper, with the ends fastened to the branch with a band of nurseryman's tape. This method would retain the moisture and prevent for some time desiccation of the mycelium. (2) The second method involves substituting for the mycelium disks cut from a diseased cypress lesion on which the fungus is fruiting. These disks were placed in the wounds with the spore-bearing surface in contact with the tissue. Nurseryman's tape was used to wrap these inoculation wounds. One inoculation and one control were used in each test. After the lapse of 2 months, data were taken on the inoculations. It should be stated that according to the temperature records during this time, from January 27 to March 30, 1938, the maximum temperature in Riverside had not exceeded

[1] Wagener, W. W. Coryneum canker of cypress. Science (n.s.) 67: 584. 1928.

[2] Wagener, W. W. The cypress bark canker and other cypress diseases. Third Western Shade Tree Conf. Proc.: 79–85. 1936.

[3] Cypress bark canker and the Monterey cypress. Science (n.s.) 86: 2230. Suppl. 8–9. 1937.

81° F. It was a time of moderate temperature with no excessively hot and drying periods. The trees were growing in 5-gallon cans in a lath house.

The positive results of these inoculations are summarized in tables 1 and 2. The sources from which the trees were obtained are indicated. The names

TABLE 1.—*Lesions on species of Cupressus from inoculations by Coryneum from cypress canker: 1 inoculation and 1 control in a series. S = Accession number of Rancho Santa Ana Botanic Garden. SPI = U. S. Department of Agriculture Seed and Plant Introduction number. Nursery indicates purchased from a local nursery. CES = Tree on the Citrus Experiment Station grounds. TL = Seed came from type locality*

Host species	Source of hosts	Diameter of stem inoculated	Radius of lesion after 2 mo.	Length of lesion after 4 mo.	Amount of stem girdled
		mm.	*mm.*	*mm.*	
arizonica	S 2357	10	20	60	$\frac{1}{2}$
arizonica	S 2358	12	10	50	$\frac{1}{2}$
arizonica	Seedlings	15–17	10–15–20	15–30	$\frac{1}{4}$–$\frac{2}{3}$
bakeri TL	S 2127	10	15	90	all
duttoni TL	S 2157	12	15	35	$\frac{1}{2}$
forbesi	S 2315	15	20	45	$\frac{2}{3}$
forbesi	S 2319	15	15	60	$\frac{1}{2}$
forbesi TL	S 2335	10	15	45	$\frac{2}{3}$
glabra	CES	20	30	70	$\frac{1}{2}$
goveniana	S 2181	12	30	60	all
goveniana TL	S 2182	14	20	50	all
guadalupensis	Nursery	20	15	30	$\frac{1}{2}$
lusitanica	SPI 73844	30	30	90	$\frac{1}{2}$
macnabiana	S 2154	10	15	30	all
macnabiana	S 2118	10	30	45	all
macrocarpa	S 2177	6	30	50	all
macrocarpa TL	S 2184	15	30	70	all
pygmaea TL	S 2133	15	25	70	$\frac{1}{2}$
pygmaea	S 2137	11	35	78	$\frac{3}{4}$
sargenti	S 2185	15	20	60	$\frac{2}{3}$
sargenti	S 2167	7	20	90	all
sargenti TL	S 2156	10	20	70	all
sempervirens	CES	12	25	40	$\frac{1}{2}$
thurifera	S 2356	17	22	70	$\frac{2}{3}$

TABLE 2.—*Lesion produced 2 months after inoculation on species of the Cupressaceae by Coryneum from cypress: 1 inoculation and 1 control in a series. Nursery = Purchase from local nursery. SPI = U. S. Department of Agriculture Seed and Plant Introduction number. CES = Tree on campus of Citrus Experiment Station*

Host species	Source of hosts	Maximum radius of lesion in mm.
Thuja plicata	Nursery	15
T. orientalis	CES	30
T. occidentalis	Nursery	20
Juniperus cedrus	SPI 57080	20
J. californica	CES	20
J. phoenicea	SPI 65020	0
J. procera	SPI 27505	0
J. procera	SPI 60553	0
J. virginiana	Nursery	10
Librocedrus decurrens	"	10
Chamaecyparis lawsoniana	CES	0

and identification number are those of the institution from which the material was received. An examination of the inoculations on *Cupressus* spp. after 3.5 months (1.5 months, since taking the data listed in Table 1) shows a marked advance of the lesions around the inoculated stems, which are now ⅓ to ⅘ girdled, with a few stems entirely so. The spread of the disease lesion on the other inoculated species of the family Cupressaceae has not been so rapid. The cypress *Coryneum* is apparently parasitic through wounds on many species of a number of genera of the family Cupressaceae (*Cupressus, Juniperus,* and *Thuja*). It probably is true that, under natural conditions, some of these species may not prove to be susceptible to the disease.

Inoculations in the following species of conifers in these experiments gave negative results: *Pseudotsuga taxifolia, Taxus baccata, Ginkgo biloba, Cryptomeria elegans, C. japonica, Araucaria bidwilli, Pinus halepensis, Taxodium mucronatum, Sequoia gigantea, S. sempervirens, Larix europea,* and *L. leptolepsis.*

Inoculations made on rose, peach, oak (*Quercus lobata*), elm (*Ulmus parvifolia*), and walnut (*Juglans regia*) gave negative results.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

A Technique for Studying the Longevity of Phoma lingam in the Soil.—When visiting the University of California in 1936, I learned from Dr. P. A. Ark of his technique for detecting the presence of *Bacillus amylovorus* in soils. Injured immature pears were suspended for 15 minutes in an aqueous suspension of the suspected soil. If viable organisms were present fire blight developed on the pears.

On my return to New Zealand, experiments were made to ascertain whether the method could be used to demonstrate the presence of *Phoma lingam* in soil. Roots of Superlative swedes were topped, washed, and incised with a sterile scalpel. In groups of 4 they were immersed in one of a series of aqueous spore suspensions containing from one spore per 10 cc. to 100,000 spores per 1 cc. Dry-rot lesions, typical of those caused by *P. lingam,* developed on swedes steeped in suspensions containing 5 or more spores per cc. (Table 1 and Figure 1, A–E).

When infection was heavy (as was caused by the suspension containing 100,000 spores per cc.) fructifications of the fungus appeared on swedes within 12 days. Normally *P. lingam* is slow to fruit, 6 weeks being required when inoculations are made from agar cultures on swedes, and 2 months on potato-dextrose agar cultures incubated at 21° C.

To test the application of the method in the field, aqueous suspensions were made with soil collected within a foot of infected swedes and also with soil taken from the same places 2 months after the removal of the swedes. Roots steeped in both suspensions became heavily infected with *Phoma lingam* (Fig. 1, F).

TABLE 1.—*Dry rot infection of swedes 10 days after submergence in aqueous suspensions of spores*

Strength of suspension	Number of wounds on 4 roots		Percentage of wounds infected
	Infected	Healthy	
0.1 spore per cc.	0	58	0
5 spores " "	2	71	3
50 " " "	44	35	56
100 " " "	24	49	33
500 " " "	56	3	93
1000 " " "	55	7	89
10,000 " " "	48	5	91
100,000 " " "	55	0	100
Check	0	62	0
Steeped in soil suspension	42	3	93

FIG. 1. Injured swedes. A. Check steeped in sterile water. B–E. Steeped in suspensions of *Phoma lingam* spores containing respectively: B, 50 spores per cc.; C, 100 spores per cc.; D, 500 spores per cc.; E, 100,000 spores per cc. F. Steeped in soil suspension.

The method facilitates studies on the longevity of *Phoma lingam* in the soil, a subject previously proved difficult owing to the numerous contaminations that occur in dilution plates and the slow growth of the fungus in culture.—J. G. GIBBS, Plant Research Bureau, New Zealand Department of Scientific and Industrial Research, Palmerston North, New Zealand.

The Longevity of Ceratostomella ulmi in Soils.—In the eradication program against the Dutch elm disease, the question of the growth and survival

of the causal fungus, *Ceratostomella ulmi* (Schwarz) Buisman, in soils was raised as one that might have a decided influence on the program. Infected roots and stumps in many cases had to be left when diseased trees were destroyed, and, if the causal organism spread from these and other sources into the soil and survived for any considerable time, the control program might be seriously affected.

Attempts were made to isolate *Ceratostomella ulmi* from soils around diseased stumps in nature. Many of these attempts were made from soil in contact with diseased wood from which *C. ulmi* was isolated at the time of soil sampling. Soil samples taken near the root collar were in contact with debarked wood, while those taken near small lateral roots were in contact with the bark only that in some cases was alive and in others dead. Eight stumps under various conditions of soil and exposure were used and soil cultures were made several times during the spring of 1936. No *C. ulmi* was isolated from any of these soil samples.

In order to get better sampling, 6 live, diseased stumps of naturally infected trees were placed, in the spring of 1936, in a sandy loam soil in 6 concrete bins constructed with drains for collecting run-off water. The soil was kept moist by artificial watering when necessary. After the stumps had been in place 30 to 60 days, sufficient water was added to secure a liter of run-off from each bin. No *Ceratostomella ulmi* was isolated from the water. In another bin heavily infected elm stem wood was buried, and a small amount of *C. ulmi* was isolated from run-off collected after the wood had been buried for 14 days. No further isolations were attempted.

In September, 1935, 7 lots of 10 test tubes each, approximately 2 inches in diameter and 16 inches long, were partly filled, respectively, with clay loam, loam, clay, sand, swamp muck, hardwood-forest humus, and forest litter, all from the Dutch elm disease area in New Jersey. Five of the tubes of each soil type were sterilized on each of 3 consecutive days at 105°–110° C. All were then inoculated with a heavy suspension of spores of *Ceratostomella ulmi* and incubated under laboratory conditions. Throughout the experiment the tubes were kept at a constant weight by adding sterile distilled water when needed to maintain the soils in a well-moistened condition.

After 3 months, *Ceratostomella ulmi* was isolated in abundance from all the sterilized soil samples, but it was not isolated from any of the nonsterilized soils, except the forest humus and only sparingly from that. After 6 months no *C. ulmi* was isolated from the nonsterilized series, but it was recovered from the sterilized soils. At that time all the nonsterilized soils were reinoculated with *C. ulmi*, which could not be reisolated 4 months later. By that time (10 months after the original inoculations) the sterilized soils had become contaminated and *C. ulmi* was isolated from but 10 of the 35 tubes.

In another test about 30 samples each of sand, clay, rich forest humus, and elm leaves were placed in 50-cc. beakers. One-third of the samples of each soil type were steam-sterilized for 20 minutes at 15 pounds' pressure. Distilled water was added to each beaker to make 2 series of moisture contents, one for sterilized and the other for nonsterilized soils, varying for each soil

type from air-dry to supersaturated. All were then inoculated with a heavy spore suspension of *Ceratostomella ulmi.* The air-dry samples were incubated over calcium chloride in desiccators. The other samples were incubated in "desiccators" in which a saturated atmosphere was maintained. The desiccators were all stored at room temperature. *C. ulmi* developed rapidly in the sterilized series, except in air-dry soils, and an abundance of conidia and coremia formed on the surface. A few coremia also formed on the nonsterilized clay, but all macroscopic evidence of *C. ulmi* disappeared after 30 days. Coremia were still abundant in the sterilized series after 120 days. Isolations made at that time showed the fungus to be alive in all except one of the sterilized samples, but it was recovered from only one beaker of sand stored over calcium chloride in the nonsterilized samples.

When humus soil treated in 3 ways (sterilized, nonsterilized, or sterilized and subsequently reinoculated with a suspension of its previous organisms) was inoculated with *Ceratostomella ulmi,* the fungus survived only in the sterilized soils not reinoculated with soil organisms. This indicates that survival or lack of survival of *C. ulmi* in soil is dependent largely on the absence or presence of competing organisms.

In the experiments here reported isolations were made from soil suspensions poured on elm-wood chips, acidified malt or potato-sucrose agar, and potato-sucrose agar containing 3 cc. of 10 per cent of commercial formaldehyde to 400 cc. of agar. Tests have shown that if *Ceratostomella ulmi* is present, even in very small amounts, it can be detected in natural soils on at least one of the media used. Consequently, the data indicate that, even if *C. ulmi* passes into the soil, in nature, it will survive but a short period in competition with soil organisms. So far, *C. ulmi* has not been isolated from soil in nature.—A. F. VERRALL, Dutch Elm Disease Laboratory, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

BOOK REVIEW

BOYCE, JOHN SHAW. *Forest Pathology.* 1st Ed. 600 pp., illus. McGraw-Hill Book Company, New York and London. 1938. $5.00.

The book devotes itself to the diseases of forests and forest products rather than of shade trees. In territory, it covers the United States and Canada; but full use is made of pertinent European material. It is designed, primarily, for use in teaching pathology to advanced forestry students, but the author has succeeded by economy of words and careful selection of literature in making the book also an exceptionally useful work of reference; there is much information that is useful only for reference. The degree of compression of the book, as also the magnitude of the field for which the forest pathologist is responsible, is indicated by the fact that there are considered diseases of more than 200 host species. The bibliography contains more than 1300 titles, placed at the ends of the chapters to which they apply and particularly featuring papers that themselves contain bibliographies. It is well indexed, 30 pages being so employed. The numerous good illustrations are closely pertinent to the text. Following general chapters on disease and on fungi, the diseases are classified partly on the basis of cause or type and partly on the age or part of the plant infected; one of the best indications of the practical workability of this classification is in the fact that most of the literature references have important bearing only on the material of the chapter to which they are attached. The group of subjects most fully covered in the book is the decays and fungus discolorations of wood, covering 30 per cent of the text matter—an emphasis reflecting both the importance of heart rots during the harvesting of the remains of our overmature forests, and the fact

that they are relatively well known, as well as the author's intimate acquaintance with them. A noteworthy feature is the chapter on the deterioration of dead timber. Other topics appropriately featured include seedling diseases, chestnut blight, white pine blister rust on which, also, the author, himself, is an authority, and Dutch elm disease.

Although generalized discussions for each type of disease appear at the beginning of each chapter, in addition to the brief introductory chapters, the reviewer feels that somewhat more material of a general nature and a slightly more philosophic approach would have been justified, even at the expense of still further compression of the information about the individual diseases. In the introductory chapters one misses the historical background of the subject. For the orientation of the forestry student in the pathology field it would seem desirable to include in them more consideration of resistance, predisposition, variation in strains of parasites, dissemination, insect relationships, and morbid anatomy and physiology. The emphasis of the discussional portions of the book is more economic than biological. The treatment is in accord with the concept developed by E. P. Meinecke, that forest pathology must concern itself more with the stand than with the individual tree; but this fundamental principle is nowhere definitely expressed in the book. Meinecke's ground-breaking paper on evaluation of damage is unfortunately referred to in connection with the specific rust with which it dealt rather than with the methodology of damage studies that it was primarily intended to illustrate. The contribution of disease to the uncertainty of forest enterprises might receive more attention. The various disease hazards that arise as a result of the increasingly artificial conditions in the forest, such, for instance, as the change in the central and northern hardwood stands from seed origin to sprout origin, could be better brought out. The reader will fail to get an adequate picture of the achievements and limitations of the remarkable campaign in control of white pine blister rust. General plant pathologists might find interesting a statement of the position to which creosote has advanced in the front rank of fungicides, and the part played by impregnation treatment of less durable woods in averting the exhaustion of durable woods and in keeping wood in the picture for poles and railway ties. Many of these points are considered in the final chapter on principles of control, but some expansion and redistribution of material of this sort would improve the book.

The consideration of wilts does not adequately bring out the concept originated by F. C. Craighead, of the damage associated with the bark beetles of the genus *Dendroctonus*, the most destructive group of insects in American forests, as being in fact apparenly due to fungi that are absolutely dependent on the beetles for transmission.

Not as a criticism, but rather as evidence of the rapidity with which new information is being secured on forest diseases, it is noted that the book is already out of date on a number of points because of publications that have appeared since the text was sent to the publisher. The forest-pathology field is still in somewhat the state of crop-plant pathology in the days when Halsted was issuing his numerous papers on new diseases. Perhaps one of the best indications of the incompleteness of the knowledge of forest-tree diseases and the amount of new material that must still be lying near the surface, is the fact that the author finds for inclusion only a single American disease of the virus type for which transmissibility has been demonstrated. To the conditions mentioned as suspected of virus origin, might be added bird's-eye maple.

The treatment is conservative. The author indulges in little speculation of his own, though statements or recommendations with little more than speculative basis have unavoidably been accepted from the literature. There are very few statements with which it is possible to definitely disagree. Excess soil moisture is stated to deprive roots of oxygen because they cannot take it from water; it would be more in accordance with our other information to suppose that water-logging of soil causes oxygen hunger by hindering the movement of oxygen into and through the soil. The statement concerning the eradication of building rots departs from the general limitation to thoroughly practicable measures, in calling for the removal of *all* infected wood. So uncompromising a recommendation is often impracticable and unnecessary if sources of moisture are cut off and sufficient ventilation is provided.

In the control chapter native forest diseases are described as controllable to a sufficient degree by a proper carrying out of the various thinning and cutting operations that are a usual part of intensive management. Special attention is given the problem of the introduced disease. For present protection against foreign parasites quarantine is recognized as the most effective measure, for the various other ways of combatting introduced parasites, which are available for crop plants, are difficult or impossible to apply under forest conditions. The need for more information on diseases whose introduction may be feared is properly emphasized, as requiring a study of American species planted abroad. It might be further pointed out that most of the danger is from Asiatic fungi, in view of the large number of Asiatic tree species related to ours, and that so few American trees have been planted in Asia that special plantings would have to be made there for disease observation.

The book, in general, is a highly valuable addition to the working equipment of both pathologists and foresters. At the same time it furnishes a good summary of accomplishments in a field that has received concentrated attention during only the last 30 years.—CARL HARTLEY, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

ANNOUNCEMENT

Abstracts of Plant Science. The trustees of BIOLOGICAL ABSTRACTS have adopted a new plan of publication, to begin in 1939 that is of more than passing interest to this Society. The emergency subsidy plan in effect in 1938 will be abandoned in favor of self-support. As heretofore, there will be monthly issues covering the whole life-science field. The aim is increasing promptness, a lag of not over two or three months between printed article and printed abstract, and the annual analytical subject index in the spring. In addition to the complete edition, the monthly issues will be divided up into separately bound sections, available at lower prices, each covering closely related subject-matter divisions. The full annual index goes to all, thus still preserving to sectional subscribers one of the most important long-range features of the whole service wherever there is access to libraries—which, naturally, will want to subscribe to BIOLOGICAL ABSTRACTS complete. Thus, without destroying its integrity, the publication is made available to the different groups of biologists in a form that provides many of the advantages they would enjoy if each society had its own abstracting journal.

The following sections will be available in 1939:

I. *Abstracts of General Biology* to include General Biology, Biography-History, Bibliography, Evolution, Cytology, Genetics, Biometry, and Ecology; price $4.

II. *Abstracts of Experimental Animal Biology* to include Animal Physiology, Nutrition, Pharmacology, Pathology, Anatomy, Embryology, and Animal Production; $9.

III. *Abstracts of Microbiology and Parasitology* to include Immunology, Bacteriology, Viruses, Parasitology, Protozoology, and Helminthology; $5.

IV. *Abstracts of Plant Sciences* to include Phytopathology (with plant viruses), Plant Physiology, Plant Anatomy, Paleobotany, Systematic Botany, Agronomy, Horticulture, Forestry, Pharmacognosy, and Pharmaceutical Botany; $6.

V. *Abstracts of Animal Sciences* to include Paleozoology, Parasitology, Protozoology, and Helminthology, Systematic Zoology, and Economic Entomology; $6.

The price of the complete volume 13, for 1939, will be $25 to individual subscribers and institutions alike.

It was with regret that the trustees, after careful study, found that, in the absence of subsidy, personal subscriptions cannot be offered at a reduction, as heretofore. At the uniform prices set, it will require something like 1250 subscriptions to the complete edition and four times as many to the sections to insure publication without deficit. The libraries will doubtless take care of the former. It is expected that the usefulness and economy of the section

plan will insure the latter. As in the past, members of societies contributing $2 or more per person to BIOLOGICAL ABTRACTS will be entitled to (a) deduct this from subscription price or (b) receive the complete index.

Plant pathologists will be interested particularly in Section IV, ABSTRACTS OF PLANT SCIENCE. Subscription blanks will be sent shortly to members of all societies represented in the Union of American Biological Societies. It would be appreciated if subscriptions could be sent in at once to BIOLOGICAL ABSTRACTS, c/o University of Pennsylvania, Philadelphia, Pa., in order that the management may receive an early indication of the number to be provided for.

It is necessary to add that the Board sees no other plan possible for the continued publication of BIOLOGICAL ABSTRACTS, since it should and must be fully self-supporting hereafter. This will be possible if the volume of sectional subscriptions promptly reaches the expected level.

The American Phytopathological Society
Committee on Biological Abstracts.

THE RELATION OF NITROGEN NUTRITION TO VIRULENCE IN PHYTOMONAS STEWARTI

GEORGE L. MCNEW

(Accepted for publication July 25, 1938)

Although the pathological relationships and physiological characteristics of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* have been determined by extensive studies, very little is known about the physiological processes that enable the bacteria to invade tracheal tubes of maize and cause wilting. Some of the strains of this bacterium, isolated from infested soil (4), infected plants (2, 3, 13), and pure cultures (8) have been reported to differ in virulence. Several such strains were tested recently in order to gain information on the physiological characteristics associated with virulence. It was found that, while the strains were similar in most respects, some of them differed in their nitrogen nutrition. Data on these similarities and differences and on the possible relationship between virulence and nitrogen nutrition are presented in this paper.

MATERIALS AND METHODS

The virulence of cultures was determined by inoculating sets of 15 to 20 sweet-corn seedlings of the variety Golden Bantam with bacteria from a 2- to 3-day-old broth subculture and observing the severity of infection about 10 days later. Differences in virulence are expressed by an infection index, calculated as the average number of necrotic lesions per leaf. The method for determining this index has been described in detail elsewhere (8).

The source and relative virulence at the time of isolation of the strains of *Phytomonas stewarti* used in these studies were as follows:

B–11. A virulent culture isolated from a naturally-infected Golden Bantam corn plant (8). This isolate produced many necrotic lesions and killed some of the invaded leaves. Index = 0.85.

B–1611. An avirulent single-colony isolate obtained from culture B–11. This strain never invaded the leaves sufficiently to cause either yellowing or necrosis of the veins. Index = 0.00.

B–1211. A slightly virulent single-colony isolate obtained from culture B–11. It invaded very few leaves of 10-day-old plants and rarely produced lesions. Index = 0.04.

B–1111. A slightly virulent single-colony isolate obtained from culture B–11. This strain was similar to B–1211, but it invaded a few more leaf veins. Index = 0.11.

B–1411. A virulent strain obtained from B–1111 by a series of single-colony isolations and selection of the most virulent isolate present. It produced many necrotic lesions but wilted very few leaves. Index = 0.64.

B–1011. A highly virulent single-colony isolate obtained from culture B–11

at the same time as B–1211. This strain produced large, necrotic lesions and killed many of the invaded leaves. Index = 1.20.

B–211. A weakly virulent single-colony isolate obtained from a Golden Bantam corn plant inoculated with B–1011. The plant was grown in washed sand in a greenhouse and supplied with a nutrient solution deficient in nitrogen (12). This isolate caused a few, small necrotic lesions but did not wilt the leaves. Index = 0.41.

B–311. A highly virulent strain obtained under the same conditions as B–211, except that the host had received nitrogen at a concentration optimum for plant growth (12). This isolate produced large necrotic lesions and killed about half of the invaded leaves. Index = 1.62.

B–92. A weakly virulent culture isolated by Wellhausen (13) from a wilt-susceptible inbred line of maize designated as GB. This strain invaded the leaves and produced lesions but did not cause wilting. Index = 0.67.

B–91. A highly virulent culture isolated by Wellhausen from a resistant inbred line of maize designated as OSF. It produced large necrotic lesions and killed many of the invaded leaves. Index = 1.76. B–91 and B–92 developed from the same parent culture.

The strains were maintained on nutrient-dextrose agar slants at 8° C. for a year and a half. Although the cultures were transferred to fresh media every 2 or 3 months, none of them changed appreciably in virulence, as was shown by inoculation tests (Table 1) conducted at the termination

TABLE 1.—*The average number of necrotic lesions per leaf and green weights of sweet corn seedlings inoculated with 10 strains of Phytomonas stewarti*

Strain tested	No. of lesions per leaf						Average green weight per plant gm.
	Replication number					Average	
	1	2	3	4	5		
B–1611	.00	.00	.00	.00	.00	.00 ± .00[a]	14.7 ± 1.0[a]
B–1211	.06	.04	.04	.01	.08	.05 ± .02	14.0 ± 1.7
B–1111	.13	.14	.16	.10	.10	.12 ± .03	13.5 ± 3.2
B–1411	.70	.74	.57	.71	1.06	.76 ± .18	9.1 ± 1.0
B–11	.77	.85	.80	.86	.99	.85 ± .08	12.3 ± 1.7
B–1011	.93	1.12	1.26	1.06	1.22	1.12 ± .15	11.4 ± 2.5
B–211	.52	.42	.64	.49	.49	.51 ± .08	13.7 ± 1.6
B–311	1.24	1.37	1.67	1.41	1.49	1.44 ± .16	8.2 ± 1.6
B–92	.54	.59	.72	.79	.57	.64 ± .11	12.5 ± 1.6
B–91	1.47	1.50	1.87	1.78	1.53	1.63 ± .19	6.3 ± 1.5
Uninoculated controls	.00	.00	.00	.00	.00	.00 ± .00	16.3 ± 1.7

[a] Standard deviation of the mean.

of the experiments to be reported. The data presented in table 1 show that the infection index is reliable to within about .05 of a lesion in determining differences between slightly virulent strains, but only to within about .30 of a lesion for the more virulent strains.

Some of the strains were analyzed for variants. The dilution-plate method was used for separating the variants. It has been shown (9) that practically all of the colonies of *Phytomonas stewarti* in properly prepared dilution plates develop from isolated, single cells. The cultural characteristics of the strains were determined according to the methods outlined by the Committee of the Society of American Bacteriologists (11), except where otherwise noted. Hydrogen-ion determinations were made by the colorimetric method.

The standard synthetic medium used to determine the ability of strains to assimilate inorganic nitrogen contained 0.858 g. KH_2PO_4, 0.684 g. $Ca(NO_3)_2 \cdot 4H_2O$, 1.75 g. $MgSO_4 \cdot 7H_2O$, 0.37 g. $(NH_4)_2SO_4$, 15 g. dextrose, and 15 g. agar per liter of distilled water. For determining the ability to utilize different types of inorganic nitrogen, a nitrogen-deficient medium containing 0.858 g. KH_2PO_4, 1.75 g. $MgSO_4 \cdot 7H_2O$, 0.426 g. $CaCl_2 \cdot 2H_2O$, 15 g. dextrose, and 15 g. agar per liter was supplemented with either $(NH_4)_2SO_4$, $NaNO_3$, or NH_4NO_3 at concentrations sufficient to add 1 mg. of nitrogen per cc. of solution. The salts and sugars were dissolved separately, added to melted agar, adjusted to pH 6.8 to 7.0, and autoclaved for 15 minutes at 20 pounds, pressure. Some of the media were supplemented with amino acids which were obtained from the Eastman Kodak Co. except where otherwise noted.

Tests for physiological characteristics of the strains were made in duplicate and repeated at least once. Inoculation tests were repeated except for those of the single-colony isolates reported in figure 3, A and B. In these 2 experiments a few of the most virulent and least virulent isolates in each group were tested a second time. The results obtained agreed closely with those reported.

Seedlings grown in washed sand and supplied with nutrient solutions according to the method of Spencer and McNew (12) were used in studies on the relation of nitrite production to wilting. The nitrogen-deficient solution described in table 1 of their paper was supplemented with either $(NH_4)_2SO_4$, $NaNO_3$, or NH_4NO_3 at a concentration equivalent to 1 mg. of nitrogen per cc. of nutrient solution.

EXPERIMENTAL RESULTS

Physiological Properties

The 10 strains of *Phytomonas stewarti* possessed the general characteristics attributed to this species (1), with the exceptions described below. All were small, Gram-negative, non-sporogenous, non-motile rods. None produced indol from tryptophane (Difco) or gas in nutrient broth containing

TABLE 2.—*Final pH produced by 7 strains of Phytomonas stewarti after 12 days' incubation at 26° C. in broth containing different carbohydrates*

Carbohydrate in media	pH of media inoculated with culture														pH of uninoculated media	
	B-1211		B-1111		B-1411		B-11		B-1011		B-91		B-92			
Maltose	6.9	6.9	6.9	6.9	6.9	6.8	7.1	7.1	7.1	7.1	7.1	7.1	7.0	7.0	7.0	7.0
Sucrose	5.5	5.7	5.5	5.5	5.5	5.7	5.7	5.7	5.3	5.5	5.5	5.5	5.5	5.5	6.9	6.9
Levulose	5.7	5.7	5.6	5.6	5.6	5.6	5.7	5.7	5.6	5.6	5.6	5.6	5.5	5.5	6.5	6.5
Dextrose	5.3	5.5	5.7	5.5	5.5	5.3	5.3	5.3	5.3	5.4	5.3	5.3	5.3	5.5	6.8	6.8
Mannose	5.7	5.7	5.6	5.6	5.7	5.7	5.7	5.7	5.6	5.6	5.6	5.6	5.6	5.5	6.9	6.9
Arabinose	5.6	5.6	5.6	5.6	5.5	5.5	5.6	5.7	5.6	5.6	5.5	5.5	5.5	5.6	6.2	6.2
Xylose	5.7	5.7	5.6	5.6	5.5	5.5	5.7	5.7	5.7	5.6	5.5	5.5	5.5	5.5	6.2	6.2
Rhamnose	7.1	7.3	7.2	7.3	7.2	7.2	7.3	7.3	7.3	7.3	7.3	7.3	7.2	7.2	6.7	6.7
Dulcitol	8.4	8.4	8.4	8.4	8.2	8.3	8.4	8.4	8.3	8.3	8.2	8.2	8.3	8.2	7.0	7.0
Mannitol	6.3	6.3	6.0	5.9	5.9	5.9	6.2	6.2	6.3	6.3	6.1	6.1	6.2	6.1	6.9	6.9

1 per cent dextrose, levulose, sucrose, maltose, mannose, rhamnose, arabinose, xylose, dulcitol, or mannitol. They produced similar changes in the hydrogen-ion concentration of nutrient broth containing different carbohydrates (Table 2). An acid reaction was induced in broth containing sucrose, levulose, dextrose, mannose, arabinose, xylose, and mannitol; no change was produced in maltose medium, and an alkaline reaction was produced in dulcitol and rhamnose media. B–92 and B–211 produced a firm, dark yellow type of growth in nutrient-dextrose agar poured plates. The other cultures produced smooth, spreading, pale yellow colonies. All strains produced a spreading, liquid, yellow growth on potato slants and caused a slight brown discoloration after 5 days. All produced a yellow sediment in litmus milk (Difco). In addition, B–91 produced acid and curd and finally dissolved the curd.

The virulent strains grew readily, on the standard synthetic medium, while the slightly virulent B–1211 and B–1111 failed to produce visible growth. During subsequent tests, it was observed that these 2 cultures may rarely produce small isolated colonies along the inoculation streak. As a general rule, however, the slightly virulent strains failed to grow either on the standard or other synthetic media (Fig. 1). The avirulent B–1611 grew as readily on the synthetic media as the virulent strains. Of the cultures that grew on the standard medium, B–91 was the only one that produced nitrites.

The standard synthetic medium was supplemented with several organic materials in an attempt to determine what nutrients were lacking for the slightly virulent strains B–1211 and B–1111. The standard medium without dextrose was supplemented with 1 per cent (except as otherwise noted) dextrose, sucrose, glycerine (5 per cent), mannitol, starch, dextrin, salicin, lactose, nutrient broth (Difco, 0.8 per cent), peptone (Fairchild's), or tryptophane. The synthetic medium with dextrose was supplemented with 1 per cent peptone, *l*-glutamic acid, *d*, *l*-phenylalanine (Pfanstiehl), aspartic acid, tyrosine, glycine, *d*, *l*-alanine, *d*, *l*-valine, egg yolk, egg albumen, casein, gelatine, urea, or asparagine (Eimer and Amend). The 2 slightly virulent strains failed to grow on any of these media, except those containing organic nitrogenous supplements (Fig. 2). Most of the amino acids proved to be less nutritive than peptone, egg yolk, or nutrient extract (Table 3). The virulent strains grew on all media except those containing only starch, dextrin, salicin, or lactose as a carbohydrate source. These strains were inhibited by phenylalanine, alanine, and valine, and slightly inhibited by urea at the concentration used.

The data show that, although the strains were similar in most respects, they differed in their ability to assimilate nitrogen. Two of the less virulent strains assimilated only nitrogen from organic sources, the strains of intermediate virulence assimilated nitrogen from inorganic salts without reducing nitrates, and the most virulent strains tested reduced nitrates to nitrites.

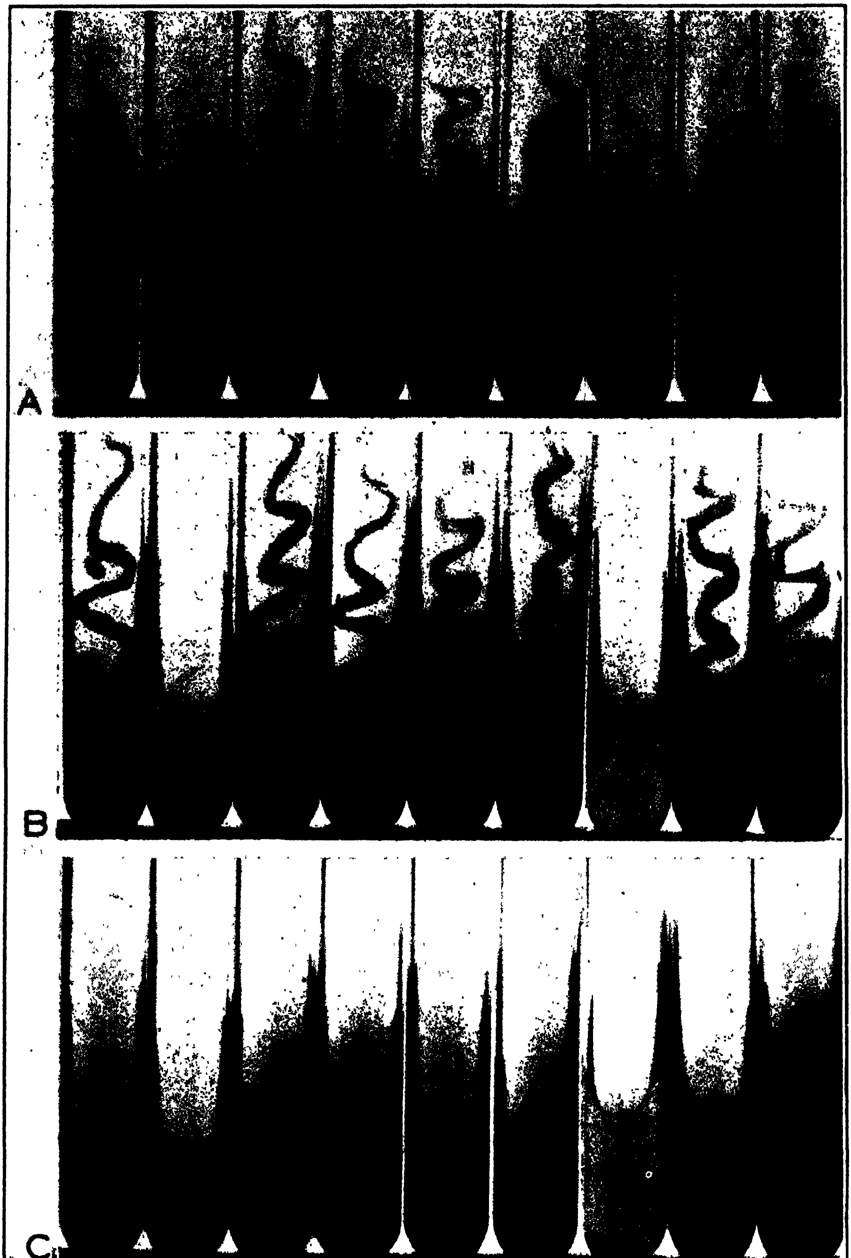

FIG. 1. Growth of highly virulent strain B-1011 (A), weakly virulent B-211 (B), and slightly virulent B-1211 (C), on different synthetic media. From left to right, the tubes contain dextrose agar with the following salt solutions: Standard medium; nitrogen-deficient medium supplemented with sufficient NH_4NO_3 to add 0.0, 0.5, 1.0, 2.0, and 4.0 mg. of nitrogen per cubic centimeter; and phosphorus-deficient solution (.558 g. K_2SO_4, .684 g. $Ca(NO_3)_2 \cdot 4H_2O$, 1.75 g. $MgSO_4 \cdot 7H_2O$, .370 g. $(NH_4)_2SO_4$ per liter) supplemented with sufficient NaH_2PO_4 to add 0.0, 0.5, and 1.0 mg. of phosphorus per cubic centimeter, respectively. The slightly virulent strain grew poorly, if at all, on all of these media, while the more virulent strains grew readily on all except those deficient in nitrogen and phosphorus. (Photographs by J. A. Carlile.)

TABLE 3.—*Growth of 7 strains of Phytomonas stewarti on synthetic agar medium with and without organic supplements*

Organic supplement		Relative development of different strains[a]						
Nitrogen	Carbohydrate	B–1211	B–1111	B–1411	B–11	B–1011	B–91	B–92
None	Dextrose	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
"	Sucrose	–, –	–, –	+, tr	++, ++	++, ++	++, ++	++, ++
"	Glycerine	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
"	Mannitol	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
"	Starch	–, –	–, –	–, –	–, –	–, –	–, –	–, –
"	Dextrin	–, –	–, –	–, –	–, tr	tr, tr	–, –	tr, tr
"	Salicin	–, –	–, –	–, –	–, –	–, –	–, –	–, –
"	Lactose	–, –	–, –	–, –	–, –	–, –	–, –	–, –
Nutrient broth	None	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++
Peptone	"	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++
Tryptophane	"	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++
None	"	–, –	–, –	–, –	–, –	–, –	–, –	–, –
Peptone	Dextrose	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++
Glutamic acid	"	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
Phenylalanine	"	–, –	–, –	–, –	–, –	–, –	–, –	–, –
Aspartic acid	"	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
Tyrosine	"	+, –	+, tr	+, +	++, ++	++, ++	++, ++	++, ++
Glycine	"	–, –	–, –	+, +	++, ++	++, ++	++, ++	++, ++
Alanine	"	–, –	–, –	–, –	–, –	–, tr	++, ++	–, +
Valine	"	–, –	–, –	+, +	+, +	+, –	+, +	+, +
Egg yolk	"	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++	++, ++
Egg albumen	"	+, +	tr, tr	+, +	++, ++	++, ++	++, ++	++, ++
Casein	"	tr, tr	+, +	+, +	++, ++	++, ++	++, ++	++, ++
Gelatine	"	+, ++	+, +	+, +	++, ++	++, ++	++, ++	++, ++
Urea	"	–, –	–, –	+, tr	+, +	+, +	++, ++	++, ++
Asparagine	"	–, tr	tr, +	+, +	+, +	++, ++	++, ++	++, ++

[a] –, no growth; tr, isolated spots; +, faint streak; ++, abundant growth.

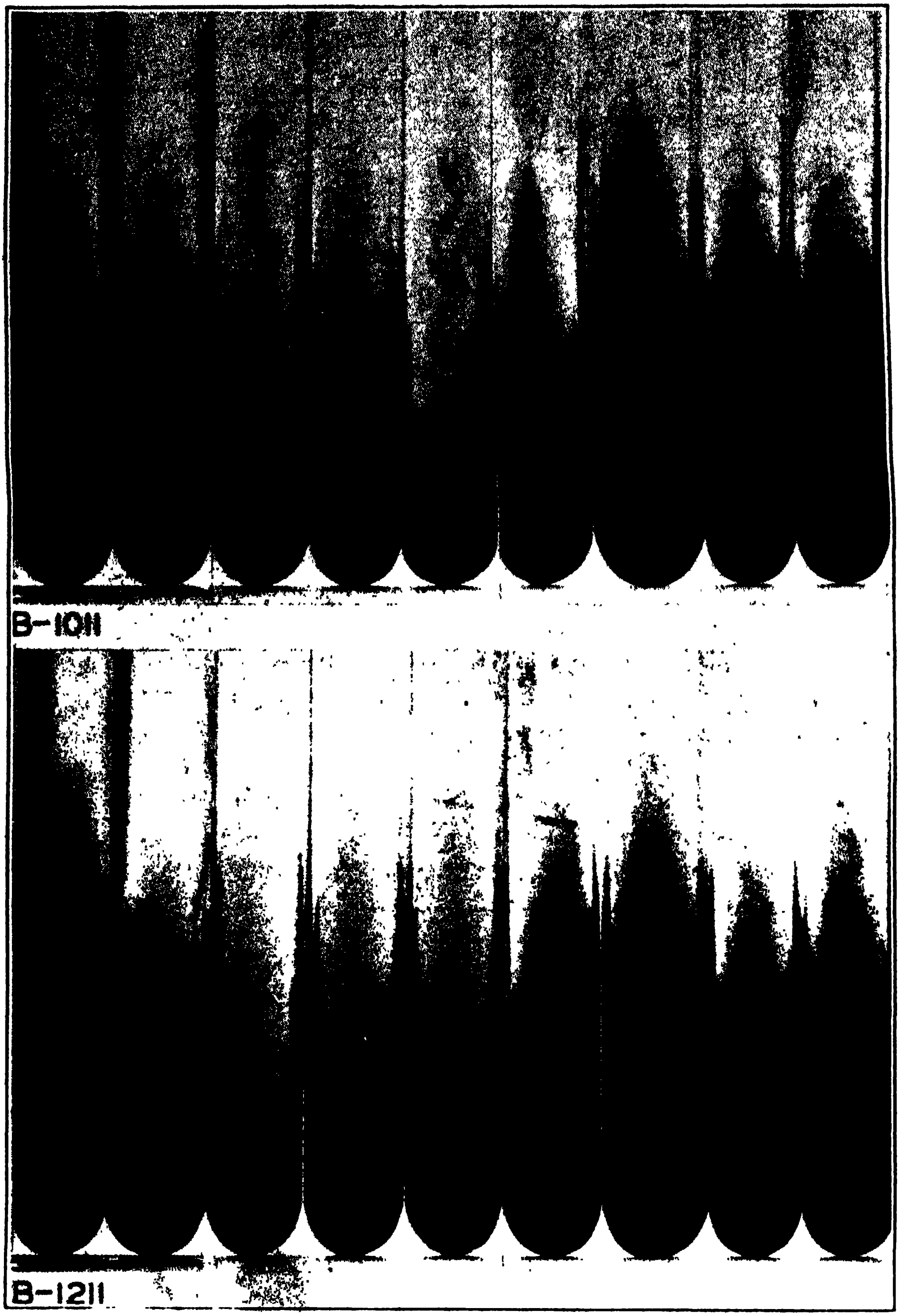

FIG. 2. Growth of the highly virulent strain B–1011 and the slightly virulent B–1211 on synthetic agar containing different organic supplements. From left to right, the tubes contain as supplements to the salt solutions: Dextrose, sucrose, glycerine, lactose, mannitol, dextrose and peptone, nutrient broth, peptone, and tryptophane. The slightly virulent culture grew only on those media that contained organic nitrogen. (Photographs by J. A. Carlile.)

Correlation of Invasiveness with Ability to Assimilate Inorganic Nitrogen

Since *Phytomonas stewarti* is a vascular parasite, it probably secures its nutrients from the transpiration stream. It seemed possible, therefore, that the differences in virulence between strains might be explained by differences in their ability to assimilate inorganic nitrogen. For this reason, experiments were made to determine whether *P. stewarti* must be capable of using inorganic nitrogen before it can invade maize and whether the nitrites produced by some strains were responsible for the severe wilting observed.

The possibility that cultures must be able to use inorganic nitrogen before they can invade the tracheal tubes was tested first. Experiments were designed to determine (1) whether all virulent cultures use inorganic nitrogen, (2) whether slightly virulent cultures regain the ability to use inorganic nitrogen when they are restored to full virulence by host passage, and (3) whether slightly virulent cultures become more virulent as they regain the ability to use inorganic nitrogen.

In the first experiment, 757 single-colony isolates from the virulent strains described in this paper and elsewhere (8) were tested for virulence and ability to use inorganic nitrogen. All of the virulent and highly virulent and some of the slightly virulent isolates grew on the synthetic medium. Only one culture failed to grow; it was found to be slightly virulent. These observations suggested that the ability to utilize inorganic nitrogen is characteristic of virulent strains.

The procedure in the second experiment was to restore culture B–1211 to virulence by host passage and then to test single-colony isolates from both the original culture and the inoculated plants for ability to grow on synthetic media. A sample from a broth subculture of B–1211 was mixed with melted agar for dilution plates before the remainder was injected into 30-day-old plants. Forty-two days after inoculation, tissues from a small, yellow leaf lesion and from the stems of 3 systemically invaded plants were macerated, diluted in broth, and suspended in melted agar for dilution plates. Fifty single-colony isolates from the broth subculture and 40 from each of the 3 plant tissues were tested for virulence. The data obtained (Fig. 3, A) show that the subculture of B–1211 contained variants, but that none of them had an index of more than 0.20. The isolates recovered from the leaf lesion were only slightly more virulent than the bacteria injected. Most of those recovered from the stems were fully virulent. They had indexes ranging from 0.10 to 1.30, with most of them between 0.40 and 1.00. All of the isolates recovered from the broth culture and the leaf lesion grew poorly on the synthetic medium. The virulent isolates recovered from the stems, however, grew very readily on this medium; the entire streak was covered with bacteria within 48 hours and masses of bacteria usually collected at the base of the slants (Fig. 1). Isolates from the stem that had indexes of less than 0.45 grew poorly on the synthetic medium.

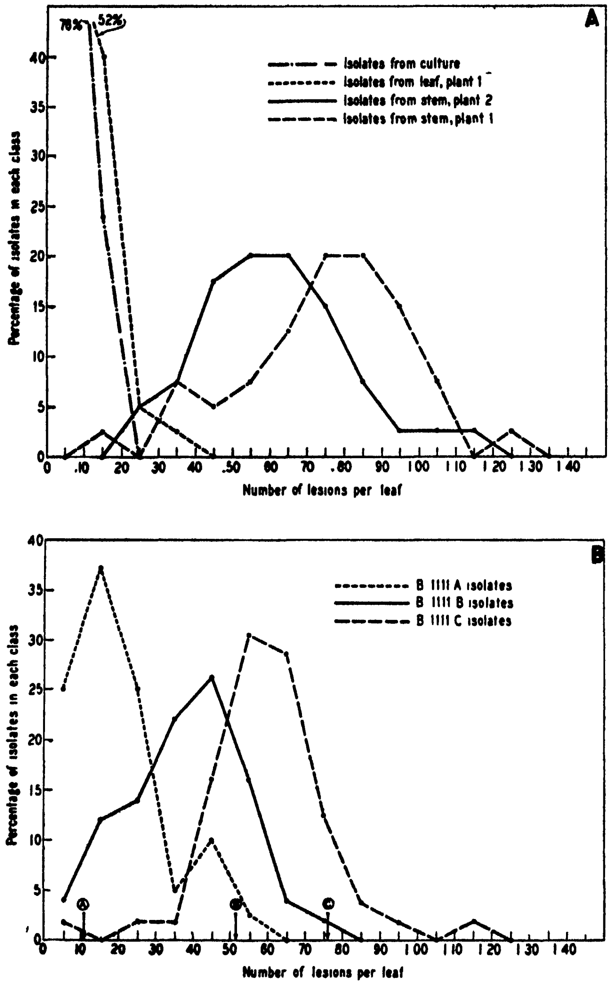

FIG. 3. Distribution of single-colony isolates from slightly virulent strains of *Phytomonas stewarti* in pathogenic classes that differ by 0.10 of a lesion each. A. The isolates recovered from the stems of sweet-corn plants inoculated by strain B-1211 were much more virulent than those obtained from either an invaded leaf vein or the culture inoculated into the plants. B. Virulent strains were obtained from culture B-1111 by pouring a series of dilution plates and selecting the most virulent isolate present. This isolate was used as inoculum for the next dilution. Isolates obtained from the third selection (B-1111 C, index of 0.76) had infection indexes that ranged from 0.00 to 1.20.

In the third experiment, cultures B-1211 and B-1111 were induced to grow on the synthetic medium and then tested for virulence. In a typical test, culture B-1211 was transferred to 80 slants of synthetic agar and several of nutrient agar. Isolated points of growth occurred on some of the synthetic slants after 5 or 6 days. Successful transfers to other synthetic agar slants were secured from only 3 of these isolated colonies. After 3 serial transfers, the cultures on synthetic agar and those on nutrient agar were transferred to tubes of nutrient-dextrose broth, incubated for 18 hours, and then tested for virulence. The data presented in table 4 show that, without exception, the

TABLE 4.—*Virulence of culture B-1211 for sweet-corn seedlings after passage through nutrient and synthetic media*

Test No.	Treatment of culture		No. of plants tested	Status of leaves			Infection index
	Media used	No. of passages		Total no.	No. wilted	No. of lesions	
I	Nutrient	3	15	75	0	1	.01
	"	3	15	77	0	2	.02
	"	3	13	70	0	1	.01
	Synthetic	3	15	76	7	39	.79
	"	3	12	58	7	34	.95
	"	3	14	65	1	42	.69
	Uninoculated controls		15	83	0	0	.00
II	Nutrient	10	15	102	0	7	.07
	Synthetic	10	15	104	1	34	.36
	"	10	15	105	2	74	.76
	"	10	15	110	0	62	.56
	Uninoculated controls		15	113	0	0	.00

cultures incubated on synthetic agar were more virulent than those on nutrient agar. Comparable results were secured in similar tests with culture B-1111 (Fig. 4).

The foregoing data show that the ability of *Phytomonas stewarti* to use inorganic nitrogen is closely correlated with virulence. All of the virulent cultures were able to use inorganic nitrogen readily, whereas the less virulent ones were not. When slightly virulent cultures were restored to full virulence either by host passage or by incubation on the synthetic medium, they regained the ability to assimilate inorganic nitrogen. The results suggest that virulence is dependent upon the ability to use inorganic nitrogen and support the hypothesis that *P. stewarti* must use inorganic nitrogen before it can secure sufficient nutrients from the transpiration stream to be fully invasive.

Selection of Virulent Strains from Slightly Virulent Cultures

The observations reported above show that the attenuated cultures were restored to virulence by incubation in the host or on the synthetic medium containing no organic nitrogen. The data offer little evidence, however, of the mechanism by which this change was brought about. An experiment was performed, therefore, to determine whether the more virulent strains were produced by a direct physiological stimulus on the individual, slightly virulent cells or merely by a differential selection of variant types by the host and the medium.

Strain B-1111, cultured on nutrient agar, was incubated in nutrient broth and then suspended in melted agar for dilution plates. Forty single-colony isolates were found to differ widely in virulence. Under the conditions

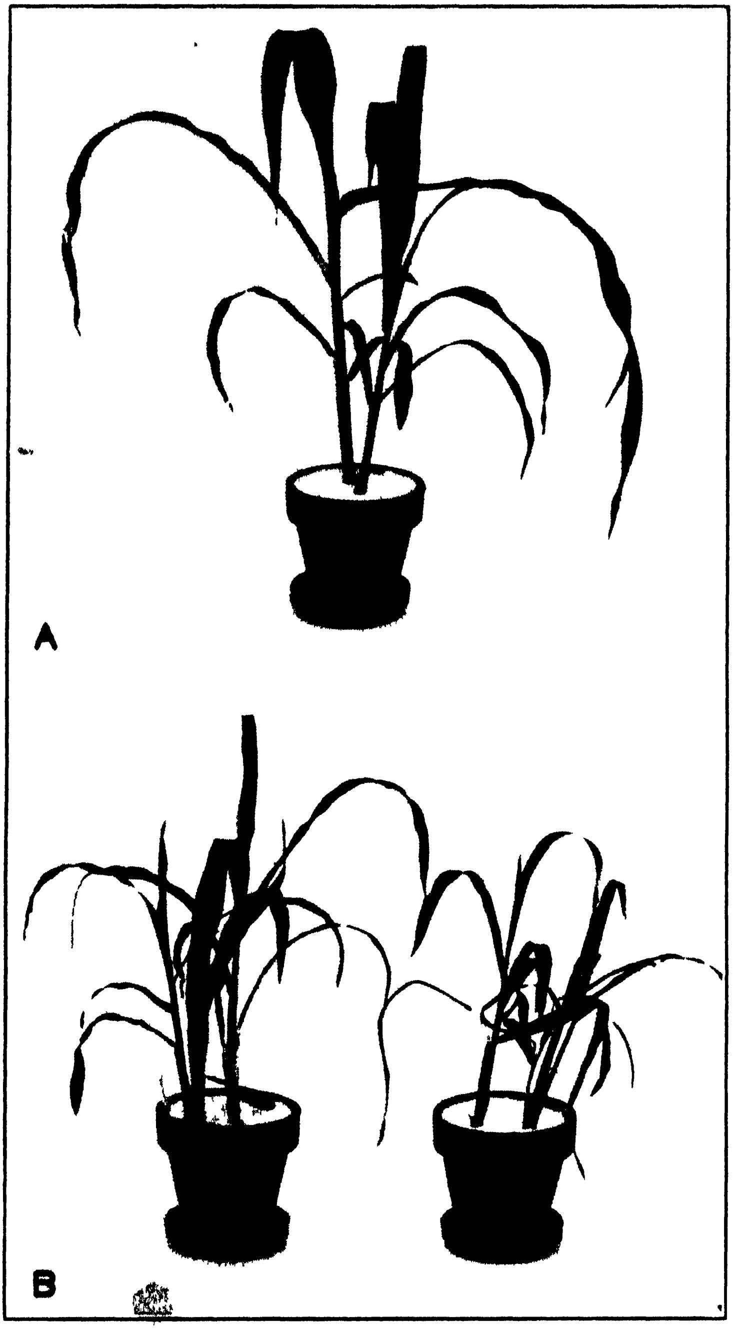

FIG. 4. Sweet-corn plants inoculated with culture B-1111 from nutrient-dextrose agar slants (A) and from synthetic agar slants (B). The 2 cultures were transferred twice on their respective media, subcultured in nutrient-dextrose broth for 18 hours, then injected into 10-day-old plants. The photographs were taken 20 days after inoculation. (Photographs by J. A. Carlile.)

of this experiment, culture B-1111 had an index of 0.11 and its most virulent strain (B–1111B) an index of 0.52 (Fig. 3, B). This strain, B–1111B, was maintained on nutrient agar slants for 28 days and then tested for variants. One of the 40 single-colony isolates obtained had an index of 0.76 (B–1111C). This isolate was, in turn, tested for variants after it had grown on nutrient agar slants for 30 days. The data presented in figure 3, B, show that 56 single-colony isolates obtained from B–1111C had indexes ranging from 0.10 to 1.20. Most of the isolates were similar to the parent, since they had indexes of 0.40 and 0.80. This experiment shows that it is possible to secure virulent cultures from slightly virulent ones by selecting the most virulent strains that are produced on an ordinary culture medium. The data also show that the restoration of virulence to the attenuated culture occurred by a number of definite steps; that is, a slightly virulent cell produced a weakly virulent one, the weakly virulent one produced a virulent one, and the virulent one finally produced others that were even more virulent. There was evidence that the new variants tended to produce many cells that reverted to the original slightly virulent parent type (B–1111).

The 56 isolates obtained from the plate poured with B–1111C were tested on a synthetic medium. All of those having infection indexes of more than 0.70 grew readily, and those with indexes of less than 0.45 grew poorly if at all. Some of the isolates of intermediate virulence (index 0.45 to 0.70) grew, while others failed to develop visible growth. Although the virulent isolates obtained from nutrient media always grew when transferred to the synthetic medium, they did not grow so readily as virulent cultures recovered from the host. They required about 1 day longer to produce visible growth and about 2 days longer to completely cover the slant than the virulent isolates obtained from the host.

Several of the isolates with indexes of 0.45 to 0.70 that grew poorly on the synthetic medium were subcultured, inoculated into seedlings, and isolated as soon as lesions were produced (4 to 6 days). The isolates grew readily on the synthetic medium. This observation suggests that these strains were invasive because they readily produced progeny that were capable of using inorganic nitrogen.

The above data show that slightly virulent strains produced variants some of which are more virulent than themselves. On ordinary nutrient agar the highly pathogenic variants constitute such a small proportion of the population that the culture, as a whole, is almost avirulent. The culture became more virulent, however, as the variants were selected out and the less virulent strains eliminated. Apparently passage either in the host or on the synthetic medium selected out the virulent types. There is little reason to assume that the bacterial cells were changed by a physiological stimulus of the host or the synthetic medium, since the same change in virulence was effected on nutrient agar by a simple mechanical selection of variants.

The Rôle of Nitrites in Producing Wilt

It having been shown above that there is a close correlation between ability

to use inorganic nitrogen and invasiveness, attention was next directed to the question of whether or not the nitrites produced by some strains (B–91) were responsible for the severe wilting observed. The wilting induced by other vascular parasites, such as species of *Fusarium,* has been attributed (10) to the nitrites they produce. In order to establish such an explanation for wilting, it would be necessary to demonstrate that highly virulent strains produce nitrites, that nitrites are produced in the severely wilted hosts, and that solutions of nitrites cause healthy plants to wilt.

Forty single-colony isolates obtained from B–91 were tested for virulence and ability to reduce nitrates, in order to determine whether or not these properties are correlated. Twelve of the isolates failed to reduce nitrates in culture. Most of these isolates were almost as virulent as those that reduced nitrates. This observation agrees with the data obtained on B–1011 and B–311. Although these 2 strains failed to reduce nitrates in culture, they were almost as virulent as B–91 (Table 1).

The above observation suggested that nitrite production is not essential for high virulence in *Phytomonas stewarti.* Further studies were made on nitrite production because it was observed that some cultures, such as B–1011, that did not produce nitrites in culture caused accumulation of nitrites in completely wilted seedlings. Leaf whorls from 10 15-day-old plants killed by B–1011 all contained nitrites, but positive tests were rarely secured from recently-formed leaf lesions. Both the leaf whorl and small leaf lesions in plants invaded by B–91 contained nitrites. Cultures B–91, B–92, and B–1011 were, therefore, tested further for ability to utilize different types of inorganic nitrogen and to reduce nitrates, in the hope of finding an explanation of why B–1011 produced nitrites in the host but not in culture. These strains were transferred to synthetic media containing either $(NH_4)_2SO_4$, NH_4NO_3 or $NaNO_3$. All the strains grew readily on the 2 media containing ammonium; but, as reported above, B–91 was the only one to reduce NH_4NO_3 to nitrites. No nitrites were produced in the $(NH_4)_2SO_4$ medium. Culture B–91 was the only strain that grew as readily on the $NaNO_3$ medium as on the ammonium-containing ones. Culture B–1011 did not produce visible growth on this medium until 5 days after inoculation, and then it was very feeble. Successful transfers were secured from some of these slants and after 2 passages on the nitrate medium the bacteria were found capable of reducing nitrates. However, such nitrate-reducing strains of B–1011 failed to reduce nitrates when subsequently transferred back to the NH_4NO_3 medium. Culture B–92 failed to grow on the $NaNO_3$ medium, even after long incubation periods. These data show that B–91 readily used either ammonium or nitrate nitrogen but that B–1011 used nitrates only when deprived of the other type of nitrogen. This observation suggests that B–1011 did not reduce nitrates in culture because there was an excess of ammonium salts, but that it was forced to utilize nitrates in the host after the supply of ammonium nitrogen was exhausted. This hypothesis is sup-

ported by the observation that B–1011 had produced no nitrites in recently invaded leaf tissue.

The nitrites produced in the plant by B–91 and B–1011 may have been responsible for some of the symptoms observed. Water-soaked streaks were produced in the leaves of healthy 8-day-old seedlings injected with 2 per cent solutions of either $NaNO_2$ or KNO_2. More concentrated solutions caused a general wilting and collapse of the plants. Solutions of either KNO_3 or $NaNO_3$ at 10 times this concentratin caused no appreciable wilting. Excised 20-day-old plants were severely wilted after 24 hours in 0.5 per cent solutions of either $NaNO_2$ or KNO_2.

Although the foregoing observations show that bacteria produce nitrites within the host and that the symptoms of nitrite toxicity resemble bacterial wilt, they do not definitely establish the causal relationship of nitrite production to wilting. The question arises as to whether the bacterium can cause wilting under conditions that prevent nitrite formation. Evidence was obtained on this question by taking advantage of the fact that the bacterium does not produce nitrites from ammonium and that the contents of the tracheal tubes may be modified by the nutrient treatment supplied to plants (7). Four sets of 30 seedlings each were grown in washed sand supplied with either a nitrogen-deficient solution or with this solution supplemented with either $(NH_4)_2SO_4$, $NaNO_3$, or NH_4NO_3. Half of the plants receiving each of these nutrient treatments were inoculated with culture B–1011. Observations made 15 days after inoculation showed that the plants deprived of nitrogen were less severely wilted than those supplied with nitrogen (Table 5). The plants supplied with ammonium nitrogen

TABLE 5.—*Virulence and nitrite production of Phytomonas stewarti in sweet corn seedlings receiving different types of nitrogen*

Treatment of plant		No. of plants tested	Status of leaves			Infection index	Total green weight gm.	Nitrite test
Nitrogen source	Inoculation		Total no.	No. wilted	No. of lesions			
NH_4NO_3	B–1011	15	68	32	50	2.15	21.5	+
NH_4NO_3	None	15	86	0	0	.00	90.0	–
$NaNO_3$	B–1011	15	70	22	60	1.80	32.0	+
$NaNO_3$	None	15	91	0	0	.00	108.0	–
$(NH_4)_2SO_4$	B–1011	15	62	27	35	1.87	15.0	–
$(NH_4)_2SO_4$	None	15	78	0	0	.00	86.0	–
None	B–1011	15	71	1	69	1.01	34.0	–
None	None	15	73	0	0	.00	60.0	–

were as severely wilted as those supplied with either nitrate or ammonium and nitrate nitrogen. Wilted plants supplied with nitrates were found to contain nitrites, but those receiving only ammonium were not. The extremely high infection indexes secured with culture B–1011 in this experiment were due to the high nitrogen levels and the poor growing conditions prevailing at the time of these tests (February, 1937). Repetition of this

experiment later in the spring gave comparable results, except that all plants were less severely wilted.

The above data show that *Phytomonas stewarti* may cause wilting without producing nitrites in detectable quantities. The nitrites ordinarily produced in diseased plants grown in soil are toxic and undoubtedly contribute towards the production of wilt symptoms, even though they are not the sole cause of wilting. The aggressiveness of strains such as B–91 that readily use either ammonium or nitrate nitrogen may be due to the fact that more nitrogen is available in the tracheal tubes for their growth than for the strains that are specialized for ammonium nitrogen.

DISCUSSION

It is a matter of common observation that most pathogenic bacteria become attenuated on culture media. The standard method for restoring virulence to such cultures is to pass them through appropriate hosts, but the exact mechanism by which the host changes the culture is not known. The data presented above suggest an explanation of what might occur during the attenuation and restoration of virulence in *Phytomonas stewarti.*

Culture B–11 was 5 times purified by dilution and single-colony isolation (8) in order to insure its single-cell origin. This culture was virulent and capable of using nitrogen from inorganic salts. The evidence obtained indicated that this type of nitrogen nutrition was essential for virulence. After several transfers on nutrient-dextrose media, B–11 produced slightly virulent strains (B–1211 and B–1111) that were incapable of using nitrogen from inorganic materials. These strains grew as well as the more virulent parent type on nutrient-dextrose media. It is possible that in time they might have replaced the parent type and caused attenuation of the culture, just as de Kruif's (5, 6) cultures of the rabbit septicemia bacillus lost virulence as the avirulent form replaced the parent type. This change was brought about artificially by separating B–1211 and B–1111 from the parent type in a poured agar plate.

When cultures B–1211 and B–1111 were injected into the host, they probably failed to grow because of the limited supply of organic nitrogen in the tracheal tubes. They did multiply in a very few leaf veins, but did not spread or cause severe injury (Fig. 3, A). The only bacteria that multiplied extensively, became systemic, and caused large lesions were the progeny of B–1211 that were capable of using inorganic nitrogen. These progeny were apparently of the same type as the variants separated by the dilution-plate method from cultures of B–1111, grown on nutrient-dextrose agar. The fact that this same type of variant could be selected on the synthetic medium offered additional evidence that the host served as a selective medium for those strains capable of using nitrogen from inorganic sources.

The foregoing discussion is based upon the hypothesis that *Phytomonas stewarti* must utilize nitrogen from inorganic sources before it can be invasive. The data presented on the close correlation of these 2 properties may be

cepted as evidence either that they are one and the same thing or that they are independent characteristics that are always inherited together. The latter suggestion would be based upon the assumption that there are genes and linkage phenomena in bacteria as well as in higher plants. Although this possibility is not disputed, it is believed that the data can be explained best upon the assumption that nitrogen nutrition conditions virulence. The ability of slightly virulent strains, such as B–1611, to utilize nitrogen from inorganic sources need not detract from the hypothesis, since the avirulence of such strains may be attributed to some deficiency other than the inability to use inorganic nitrogen. On the other hand, the causal relationship of the 2 properties would be definitely disproved if any highly virulent strain failed to use inorganic nitrogen. Such a strain was not observed in the rather extensive tests conducted.

The data obtained show that an attenuated culture regains virulence by producing a series of variants, each one of which is a little more virulent than its predecessor. With our present knowledge of heredity in bacteria there is no logical explanation of why the evolution occurs in a series of steps rather than by one change from the avirulent to fully virulent. Likewise, there is no explantion of why some of the slightly more virulent variants that are produced show no corresponding increase in ability to use nitrogen from inorganic sources. It is possible that a difference in the nitrogen nutrition of these strains could be detected by employing some other method of measuring the ability to use inorganic nitrogen. The significant point in the present thesis is that cultures restored to full virulence always regained the ability to use inorganic nitrogen.

The foregoing discussion on nitrogen nutrition applies to the early stages of invasion while the bacteria are confined to the tracheal tubes. Since these tubes are composed of dead cells, the question may be raised as to whether *Phytomonas stewarti* may be considered as a true pathogen. There seems to be no legitimate reason for not considering it as such, because the bacteria produce characteristic disease symptoms and, in the later stages of invasion, penetrate the parenchymatous tissues surrounding the leaf veins (4). The invasion of these living tissues probably is controlled by ability to produce enzymes or toxins, but such materials have not yet been identified. In addition to the nitrites produced by some strains (B–91), the bacteria probably produce other materials that injure the host cells.

SUMMARY

Ten strains of *Phytomonas stewarti* that differed in virulence were found to have the following morphological, cultural, and physiological characteristics in common. All consisted of small, Gram-negative, non-motile, non-sporogenous rods. None of them produced indol from tryptophane or gas from carbohydrates. They caused similar changes in the acidity of media containing different carbohydrates and produced the same type of growth on potato slants.

The strains differed from each other in that 2 slightly virulent ones failed to utilize nitrogen from inorganic sources, 2 other strains produced small, firm, yellow colonies on nutrient-dextrose-agar poured plates, and the most virulent strain reduced nitrates to nitrites and produced a curd in litmus milk.

A series of experiments showed that ability to use inorganic nitrogen was correlated always with virulence. All virulent cultures used inorganic nitrogen, and slightly virulent cultures regained the ability when restored to virulence by host passage. Slightly virulent cultures became more virulent after they had been induced to grow on a synthetic agar medium. These changes in virulence and physiological abilities apparently were brought about as the result of an intensive selection of variants that were produced by the slightly virulent culture. The slighly virulent strain produced variants that in turn produced other variants. By proper mechanical separation and selection of these variants on nutrient agar it was possible to derive a virulent culture from a slightly virulent one by a method analogous to that occurring in the host.

Most of the virulent strains utilized ammonium nitrogen much more readily than nitrate nitrogen. The most virulent strain tested (B-91) reduced nitrates to nitrites in a medium containing both ammonium and nitrate nitrogen. This and other virulent strains produced nitrites in severely wilted plants, but the nitrites were found not to be the sole cause of the wilting.

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VARIABILITY OF GLOMERELLA GOSSYPII

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INTRODUCTION

Variations in the cultural behavior of certain isolates of *Glomerella gossypii* (South.) Edg. were observed during an investigation on the organisms involved in damping off of cotton seedlings. These variations appeared in the original isolations, suggesting that such cultural types occur in nature. The purpose of the present paper is to report observations on the variability of this fungus in respect to cultural behavior, and pathogenicity on cotton seedlings.

STUDIES ON VARIATION IN CULTURAL BEHAVIOR

As soon as colonies of *Glomerella gossypii* were recognized in platings of diseased materials, records were made of their growth characters and single conidial isolations of each were made to individual plates of potato-dextrose agar. After the monosporous colonies had grown a few days they were placed at 8°C. for future use. In taking notes on the colonies particular attention was paid to such characters as type of aerial mycelium, color of aerial and subaerial mycelium, type of colony margin, and growth rate of the colony.

After 58 monosporous isolates finally were collected, they were removed from the low-temperature chamber and single-spore transfers of each were made to fresh plates of potato-dextrose agar. These cultures were incubated at 27°C. and, after 6 days, their cultural characters were compared and recorded. The same variant cultures observed in the original platings appeared in the respective single-spore cultures.

Of the 58 isolates 45 were alike, so far as growth behavior was concerned. These were arbitrarily referred to as "normal-type" colonies. The remaining 13 cultures differed from the normal type in one or more characters and were referred to as "variant-type" colonies.

These preliminary observations suggested that more detailed studies of the phenomenon of variation would be of interest. An experiment, therefore, was set up for the purpose of determining the extent, frequency, and magnitude of variation as observed in pure culture and under constant environmental conditions. In the experiment 27 single conidial cultures were used. Fourteen of these were of the normal type (Fig. 1, A)

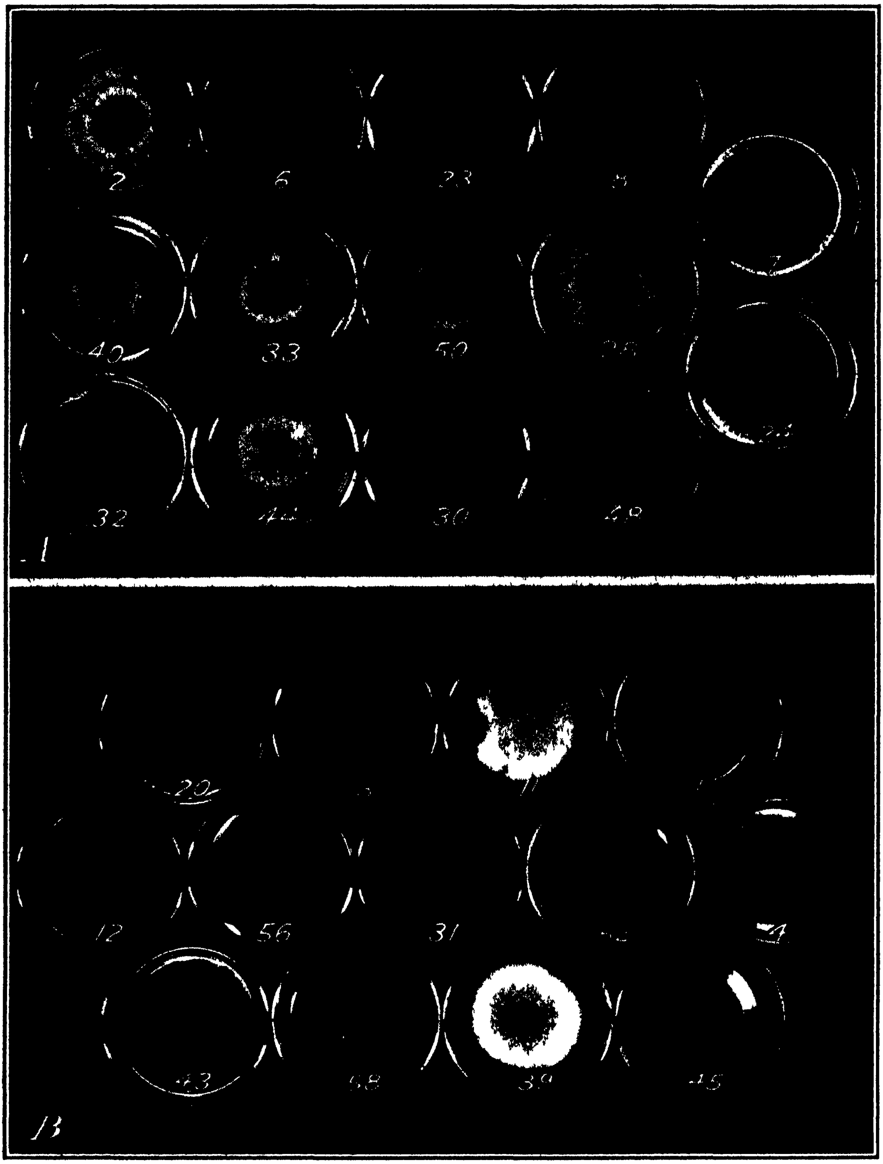

FIG. 1. Original single-conidial isolates employed in the studies on cultural behavior and pathogenicity as they appeared at the beginning of the investigation. A. "Normal-type" isolates. B. "Variant-type" isolates.

and 13 of variant types (Fig. 1, B). The history of these cultures is given in table 1.

The isolates were grown exclusively on potato-dextrose agar,[1] and all

[1] The potato-dextrose agar was made up as follows: 200 grams of peeled potatoes were placed in 500 cc. of distilled water and autoclaved for 20 minutes. The decoction was filtered and 20 grams of dextrose dissolved in it. To this 500 cc. of 4 per cent agar was added and the mixture brought up to 1 liter by the addition of water. The medium was then placed in flasks or tubes and autoclaved 20 minutes.

cultures, either on slants or in Petri plates, were incubated in the dark at 27°C. Cultures always were compared on the same batch of agar, and a uniform quantity of it was used in Petri plates (20 cc.) and slants (5 cc.). The method of handling the cultures in this experiment consisted in making weekly a transfer of each isolate by means of a spore suspension to a fresh slant. On alternate dates of transfer single-spore isolations were made from each culture in triplicate, to 3 plates of agar. When the colonies were 6 days old notes were taken on their cultural behavior and the appearance of any variant types recorded. When any of the 3 colonies isolated from a given culture showed a marked variation from the original it was transferred to a slant and the parent culture discarded.

TABLE 1.—*History of cultures of Glomerella gossypii employed in the study of variability of the causal organisms of damping off of cotton seedlings*

Culture number	Host	Origin of host	Culture number	Host	Origin of host
V[a]-1	cotton seedling	Auburn, Ga.	V-31	cotton seedling	Pendleton, S. C.
N- 2	do.	do.	N-32	do.	Auburn, Ga.
V- 4	do.	do.	N-33	do.	do.
N- 6	do.	do.	V-39	do.	Pendleton, S. C.
N- 7	do.	do.	N-40	do.	Auburn, Ga.
N- 8	do.	do.	V-43	do.	do.
V-11	do.	do.	N-44	do.	do.
V-12	do.	do.	V-45	do.	Kathwood, S. C.
V-13	do.	do.	N-48	do.	Auburn, Ga.
V-20	cotton seed	do.	N-50	do.	Pendleton, S. C.
N-23	cotton seedling	Chester, S. C.	V-52	do.	Virginia
N-24	cotton seed	Auburn, Ga.			
N-28	cotton seedling	Pendleton, S. C.	V-56	boll.	Allendale, S. C.
N-30	do.	do.	V-58	do.	Virginia

[a] V—refers to variant type in original isolation.
N—refers to normal type in original isolation.

During the course of the experiment, which involved 22 weekly transfers, all of the 27 isolates gave rise to at least 1 variant, while some produced as many as 5 distinct cultural types markedly different from one another and from the original culture. The cultures, as they appear at the end of the experiment, are shown in figure 2, A and B. As the experiment progressed the variants appeared to fall into two rather definite classes, and an additional heterogeneous class. The class containing the greatest number was characterized by a dense white aerial mycelium. The color of the subaerial mycelium and the growth rate of the cultures of this class was somewhat variable. At the completion of the experiment, cultures 4, 6, 7, 11, 12, 23, 24, 32, 39, 40, 45, 48, and 58 could be placed in this general group designated as Class I. The second class was distinguished by a sparse growth of aerial mycelium closely appressed to the surface of the agar. These colonies were

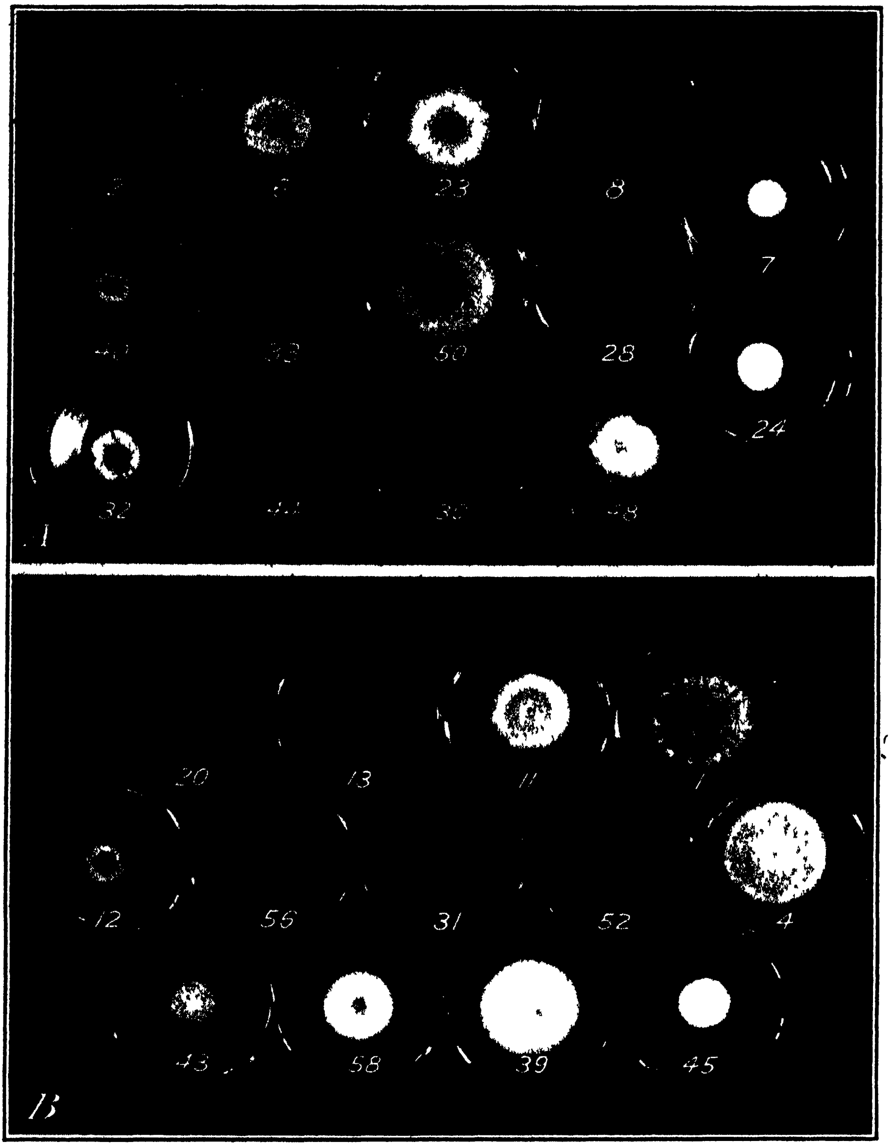

FIG. 2. Variant cultures that arose from the isolates shown in figure 1, after 22 weekly transfers. A. Variants from the "normal type" isolates. B. Variants from the "variant-type" isolates. The colonies are in the same relative positions as the parent isolates shown in figure 1.

buff with irregular, radiating streaks of gray. The cultures that at the end of the experiment could be placed in Class II were Nos. 8, 28, 30, 31, and 52. A third class comprised a heterogeneous group of cultures differing from one another and unlike either those of Class I or Class II. Cultures 1, 2, 13, 20, 33, 43, 44, 50, and 56 were placed in Class III.

When an isolate once changed in its cultural behavior from that found on first isolation, it never reverted to the original type. Variant types of

Class I never were observed to give rise to a variant type possessing the characteristics of Class II. Conversely, variants of Class II never produced variants that could be placed in Class I. Isolates of both these classes produced in turn variants of the heterogeneous type of Class III, and those of the latter class often gave rise to colonies typical of those in Class I or Class II. The variations observed could in no way be correlated with the geographical origin of the isolate nor the particular plant part from which the isolation was made. In several instances variations were in a retrogressive direction; the colonies frequently becoming smaller with each successive saltation. The great majority of variants appeared as entire colonies; few arising as sectors in the parent colony. In 2 instances the conidia of cultures V–13 and V–44 failed to grow when isolated. These cultures could be maintained only by mass transfers of large numbers of spores and mycelial fragments. Eventually, they produced other variants in which the spores grew normally on isolation. Culture V–56 gave rise to a variant completely devoid of conidia and could be propagated only by mass transfers of mycelium or hyphal-tip isolations.

CYTOLOGICAL OBSERVATIONS

Cytological preparations of the conidia were made for the purpose of observing their nuclear condition, and for obtaining some insight as to the nature of the variations under consideration.

A heavy spore suspension was spread over slides that had been smeared with egg albumen. Some of the slides were placed at once in a moist chamber to permit germination of the conidia, others were placed in a fixing solution immediately after the suspension had dried. After fixing in a 6 per cent solution of mercuric chloride containing 2 per cent glacial acetic acid, the spores were stained in Mayer's Haem-alum, dehydrated, and mounted in balsam.

Resting conidia were found to be invariably uninucleate. Although no division figures were observed, the first step in germination appears to be

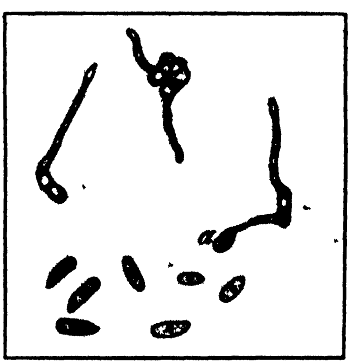

FIG. 3. Resting and germinating conidia of *Glomerella gossypii* showing the uninucleate condition in each cell. a. Appressorium. Drawings made with the aid of a camera lucida. Objective, oil immersion 95×; Ocular, 6×.

a division of the nucleus followed by the laying down of a wall in the middle of the cell and separating the two daughter nuclei. In germinating conidia each cell of the germ tubes and young hyphae contain a single nucleus (Fig. 3).

PATHOGENICITY STUDIES

The wide variations found in cultural behavior of different isolates, suggested that corresponding differences might well occur in respect to pathogenicity. Experiments, therefore, were undertaken to determine if such variations existed between the original isolates, and whether changes in cultural behavior were accompanied by changes in pathogenicity. It was also of interest to ascertain whether there was any relation between virulence and growth habit.

In a preliminary experiment, 20 of the original cultures and their respective variants were used for inoculation. The original cultures are among those shown in figure 1, A and B. These cultures were maintained at 8°C. from the time of first isolation. The corresponding variant cultures were those that appeared in the final transfer at the completion of the experiment on cultural behavior. These cultures are among those shown in figure 2, A and B.

One variety of cotton, Miller 610, was used throughout the studies on pathogenicity. Preliminary platings and germination tests indicated that this variety was relatively free of internally borne pathogenes, and gave a high percentage of germination. The seed was acid-delinted before using.

Transfers of the original isolates and their respective variants were made to flasks of potato-dextrose agar. When these cultures were incubated 5 days, a spore suspension was made in each flask with equal quantities of distilled water. A suspension of mycelial fragments was used in case of culture V–56, which failed to form spores. In each suspension 100 seeds were immersed an equal length of time and planted with sterile forceps. Controls were set up in which the seed was simply immersed in distilled water. Plantings were made in steamed river sand in flats placed on a bench in the greenhouse. The temperature of the sand was held between 20° and 25°C., and the moisture content maintained at a level that would ordinarily permit normal germination. Variations of pathogenicity, as indicated in greenhouse trials, are shown in figure 4.

Three weeks after planting, seedlings from each inoculation were removed from the sand and classified according to severity of infection. The seedlings were grouped into 4 classes:—(1) healthy, (2) lesioned, (3) post-emergence killing, and (4) pre-emergence killing.

In order to reduce the data to a more simplified form a disease index was used to express the degree of virulence shown by each culture. To obtain a disease index each class of seedlings was assigned an arbitrary value, *e.g.*, healthy = 1, with lesions = 0.5, and dead = 0. The number of seedlings falling in each class was multiplied by the respective class value,

the products added together, and the sum subtracted from 100. Thus a disease index of 100 indicates that all seedlings were killed. Pre-emergence or post-emergence killing was not distinguished by the index.

A second experiment was set up using essentially the same methods, but with fewer cultures to allow for replication. In this trial the seeds were grown in glazed 10 inch pots instead of in flats. Fifty seeds were planted in each pot and each inoculation was replicated 4 times. The pots were placed at random on a bench in the greenhouse. Culture V–56, which

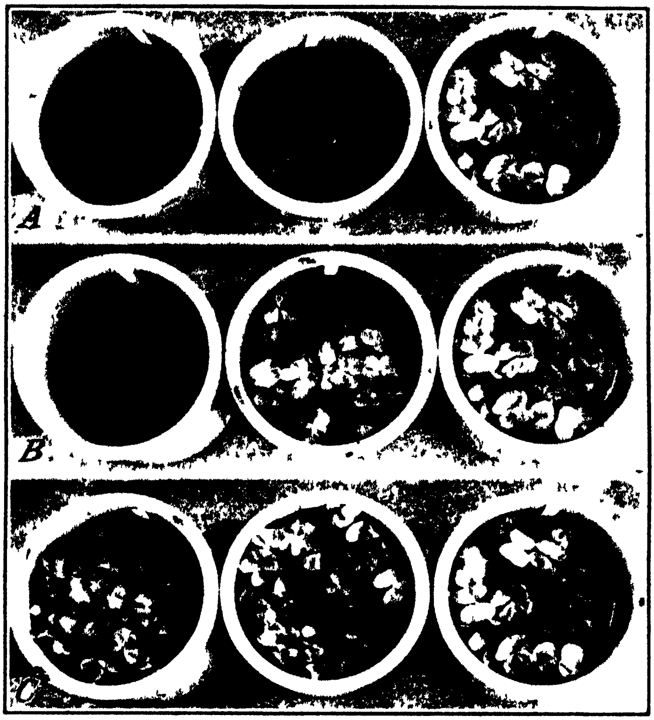

FIG. 4. Examples of differences in virulence between isolates as found in pathogenicity trials. Control plantings in each case are on the extreme right. A. Original isolate, 31S (left), and its respective variant, 31R (center), highly virulent. B. Original isolate, 7S (left), pathogenic; its corresponding variant, 7R (center), mildly pathogenic. C. Both original isolate, 52S (left), and its variant 52R (center), mildly pathogenic.

was not used in the preliminary experiment, was employed in the second trial.

The results obtained in the first experiment are shown in table 2: those of the second experiment, in table 3. A comparison of the corresponding indices in both tables indicates a close agreement between the two experiments. In the second trial, however, a higher degree of virulence was shown by all isolates. This probably was because of a lower soil temperature that prevailed while this experiment was being conducted. Isolate V–52S, the only stock culture to show low virulence, was nevertheless highly infective, as shown by the large number of lesioned seedlings. The variant

TABLE 2.—*Results of first experiment on pathogenicity, showing cultures used, class distribution of seedlings in each inoculation, disease index, and diameter of colony of each isolate used*

Treatment	Living		Dead		Disease index	Diameter of colony in cm.
	Healthy	Lesioned	Post-emergence	Pre-emergence		
Ck	86	8	0	6	10	....
"	82	12	0	6	12	....
"	78	10	0	12	18	... ...
"	84	11	0	5	11	. ...
Cult. No. N- 8S	0	0	4	96	100	8.0
" " 8R	0	0	3	97	100	8.0
" " V-20S	0	0	2	98	100	8.0
" " 20R	0	0	4	96	100	8.0
" " N-28S	0	0	4	96	100	8.0
" " 28R	0	0	4	96	100	8.0
" " N-30S	0	0	13	97	100	8.0
" " 30R	0	0	9	91	100	8.0
" " V-31S	0	0	2	98	100	8.0
" " 31R	0	0	15	85	100	8.0
" " V-52S	0	28	46	26	86	5.0
" " 52R	2	35	41	24	80.5	5.0
" " V- 4S	0	0	0	100	100	6.5
" " 4R	0	0	5	95	100	6.5
" " N- 7S	0	0	5	95	100	8.0
" " 7R	8	39	26	27	72.5	2.5
" " V-12S	0	0	5	95	100	6.0
" " 12R	0	16	15	69	92	5.0
" " N-23S	0	0	3	97	100	8.0
" " 23R	0	0	3	97	100	8.0
" " N-24S	0	0	7	93	100	8.0
" " 24R	20	43	9	28	58.5	2.5
" " V-39S	0	0	2	98	100	7.0
" " 39R	0	0	12	88	100	7.0
" " N-40S	0	0	3	97	100	8.0
" " 40R	0	10	64	26	95.0	3.0
" " V-58S	0	0	3	97	100	7.5
" " 58R	0	0	4	96	100	5.0
" " V- 1S	0	7	58	35	96.5	6.5
" " 1R	1	11	52	36	93.5	7.0
" " N- 2S	0	0	2	98	100	8.0
" " 2R	0	0	13	87	100	6.0
" " V-13S	1	0	11	88	99.0	6.5
" " 13R	0	0	6	94	100	8.0
" " V-33S	0	0	2	98	100	8.0
" " 33R	0	0	2	98	100	8.0
" " V-43S	0	0	8	92	100	6.5
" " 43R	4	52	23	21	70	5.0
" " N-44S	0	0	2	98	100	8.0
" " 44R	0	0	22	78	100	6.5

of this isolate, No. 52R, behaved in essentially the same manner. On further examination of the tables it is found that whenever wide differences in virulence exist between the original isolate and its variant, a corresponding reduction in colony diameter is shown by the latter culture. It seems very possible that growth rate is directly related to pathogenicity but is probably not the only factor involved. All divergencies in growth habit from that of the original culture were not reflected in the relative virulence of the variant. While there was some variation between cultures in respect to

TABLE 3.—*Results of second experiment on pathogenicity*

Treatment	Living		Dead		Disease index	Average disease index	Diameter of colony in cm.
	Healthy	Lesioned	Post-emergence	Pre-emergence			
Ck	35	8	4	3	22		
"	34	9	4	3	23		
"	37	4	3	6	22		
"	40	2	4	4	18		
"	38	4	2	6	20		
"	36	8	3	3	20	Ck.	
"	39	5	4	2	17	Av. = 20	
"	35	11	2	2	19		
Cult. No. N- 7S	0	1	27	22	99		
" " N- 7S	0	0	16	34	100		
" " N- 7S	0	1	19	30	99		
" " N- 7S	0	1	20	29	99	Av. 7S = 99.25	8.0
" " 7R	12	10	8	20	66		
" " 7R	23	8	3	16	46		
" " 7R	27	11	8	4	35		
" " 7R	25	9	5	11	42	Av. 7R = 47.50	2.5
" " V-39S	0	3	17	30	97		
" " V-39S	0	3	15	32	97		
" " V-39S	0	0	15	35	100		
" " V-39S	0	14	12	24	85	Av. 39S = 95.00	7.0
" " 39R	1	6	18	25	92		
" " 39R	3	6	19	22	88		
" " 39R	4	6	19	21	86		
" " 39R	2	21	14	13	76	Av. 39R = 85.50	7.0
" " N-24S	0	0	23	27	100		
" " N-24S	0	0	25	25	100		
" " N-24S	0	0	20	30	100		
" " N-24S	0	0	18	32	100	Av. 24S = 100.00	8.0
" " 24R	36	5	6	3	23		
" " 24R	30	6	9	5	34		
" " 24R	37	7	1	5	19		
" " 24R	30	12	1	7	28	Av. 24R = 26.00	2.5
" " V-56S	0	0	11	39	100		
" " V-56S	0	0	14	36	100		
" " V-56S	0	0	13	37	100		
" " V-56S	0	0	8	42	100	Av. 56S = 100.00	7.5
" " 56R	30	4	11	5	36		
" " 56R	35	6	2	7	24		
" " 56R	32	4	5	9	32		
" " 56R	35	1	6	8	21	Av. 56R = 28.25	5.5
" " N-28S	0	0	16	34	100		
" " N-28S	0	0	7	43	100		
" " N-28S	0	0	9	41	100		
" " N-28S	0	0	11	39	100	Av. 28S = 100.00	8.0
Cult. No. 28R	0	0	14	36	100		
" " 28R	0	0	18	32	100		
" " 28R	0	0	20	30	100		
" " 28R	0	0	11	39	100	Av. 28R = 100.00	8.0
" " N-30S	0	1	6	43	99		
" " N-30S	0	0	15	35	100		
" " N-30S	1	0	14	35	98		
" " N-30S	0	0	6	44	100	Av. 30S = 99.25	8.0
" " 30R	0	0	13	37	100		
" " 30R	0	0	15	35	100		
" " 30R	0	1	14	35	99		
" " 30R	0	0	17	33	100	Av. 30R = 99.90	8.0

TABLE 3.—(*Continued*)

Treatment	Living		Dead		Disease index	Average disease index	Diameter of colony in cm.
	Healthy	Lesioned	Post-emergence	Pre-emergence			
Cult. No. V–31S	0	2	27	21	98		
" " V–31S	0	0	6	43	100		
" " V–31S	0	0	15	35	100		
" " V–31S	1	0	14	35	98	Av. 31S = 99.00	8.0
" " 31R	0	0	25	25	100		
" " 31R	0	0	22	28	100		
" " 31R	0	0	18	32	100		
" " 31R	0	0	14	36	100	Av. 31R = 100.00	8.0
" " V–52S	0	35	12	3	65		
" " V–52S	3	33	10	4	61		
" " V–52S	6	24	12	8	64		
" " V–52S	5	26	13	6	64	Av. 52S = 63.5	5.0
" " 52R	1	38	9	2	60		
" " 52R	0	37	7	6	63		
" " 52R	1	33	11	5	66		
" " 52R	0	29	14	7	71	Av. 52R = 65.0	5.0
" " N–33S	0	0	11	39	100		
" " N–33S	0	0	10	40	100		
" " N–33S	0	0	7	43	100		
" " N–33S	0	0	12	38	100	Av. 33S = 100.00	8.0
" " 33R	0	0	5	45	100		
" " 33R	0	0	6	44	100		
" " 33R	0	0	6	44	100		
" " 33R	0	0	14	36	100	Av. 33R = 100.00	8.0
" " V–43S	0	1	15	34	99		
" " V–43S	0	1	30	19	99		
" " V–43S	0	0	19	31	100		
" " V–43S	0	0	24	26	100	Av. 43S = 99.5	6.5
" " 43R	10	30	5	5	50		
" " 43R	13	24	12	1	50		
" " 43R	18	16	7	9	48		
" " 43R	15	21	5	9	49	Av. 43R = 49.25	5.0

sporulation, no relationship could be found between spore production and pathogenicity.

DISCUSSION

In the studies on variation in cultural behavior it has been pointed out that differences in cultural type appeared on first isolation from diseased host tissues. Although the majority of the original isolates were of the same type, some had divergent growth habits. This suggests that variation in cultural behavior is extant in nature as well as under artificial conditions. The variants found on first isolation were not so widely divergent as those encountered in the subsequent studies.

Studies on pathogenicity of different cultures of *Glomerella gossypii* have shown that all of the original cultures, except V–52S, were highly virulent. A number of the variants that arose from the original cultures proved to be only mildly pathogenic. All variations in cultural characters from that of the original isolate were not accompanied by a reduction in virulence. The only cultural character found to be correlated with pathogenicity was that of growth rate or colony diameter.

Although wide variations have been observed in pathogenicity, it seems improbable that such variations are of great practical importance. Under natural conditions where inoculum is abundant, weakly pathogenic cultures are very likely overrun by more virulent forms.

In certain preliminary experiments not included in the present investigation it has been observed that the variety of the host plant has little or no influence on the expression of the relative virulence of any given culture, although apparent differences in varietal susceptibility were observed. This suggests that selective pathogenicity, as found in several plant pathogenes, does not exist in *Glomerella gossypii.*

The nature of variations as observed in *G. gossypii* appears to be concerned with mutation of the heritable material of the nucleus. The facts that variations occurred suddenly, were irrevertable, and tended to be in a retrogressive direction, suggest that fundamental alterations of the nuclear content are involved. Since the conidia are uninucleate it is obvious that heterocaryosis with subsequent reassortment of nuclei[2] is an untenable explanation of variation in this instance. The character of the observed variants is not that that would be expected if environmental factors were functional in bringing about such changes. It is doubtful whether the perithecial stage of this fungus, through sexual combination and segregation of heritable factors, is involved in the production of variant types in nature. Although the perfect stage has been observed in nature on one occasion,[3] no further reports of its occurrence are to be found. If the perithecia form under field conditions they probably do so only in rare instances.

SUMMARY

Variations in cultural behavior of *Glomerella gossypii* were observed on first isolation from diseased tissues, suggesting that such variant types occur in nature.

Further studies showed that all cultures employed gave rise to at least one variant during the course of the investigation. Of the variants observed the majority could be grouped into 2 rather definite classes on the basis of their growth characters. The largest class was characterized by a dense, white, cottony aerial mycelium. A second class was composed of cultures having sparse buff-color aerial mycelium.

All of the original cultures but one were highly pathogenic on cotton seedlings. Some of the variant cultures were only mildly virulent. Low virulence was directly correlated with slow growth rate of the culture.

Cytological observations revealed the conidia and hyphal cells to be uninucleate. This fact, together with the characteristic type of variation, suggests that the changes in both cultural behavior and pathogenicity are probably due to mutation of the heritable material of the nucleus.

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The variations found in cultural characters and virulence are believed to be of little importance from a practical standpoint.

DIVISION OF COTTON AND OTHER FIBER CROPS AND DISEASES,
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(In cooperation with the South Carolina Agricultural Experiment Station, Plant Pathology Section.)

A STUDY OF FORKING IN RED PINE[1, 2]

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INTRODUCTION

A high percentage of false bifurcation of the current season's growth of red pine, *Pinus resinosa* Ait., was noted in a forest planting on the eastern side of Canadice Lake, N. Y., by J. A. Brock in the spring of 1933 (9). The writer found the same disturbance in 3 counties in western New York State, in the northern Adirondacks, and in 2 counties in southeastern Pennsylvania in the summers of 1936 and 1937, although no systematic attempt was made to scout for it. H. H. York has reported it as occurring in plantings in Connecticut and several counties in central and eastern New York.

Unless specifically designed otherwise, all data included in this paper were obtained from plantings on the Rochester Municipal Watershed in Livingston and Ontario Counties, N. Y. Trees in these plantings varied from 5 to 25 years of age.

Kienholz (10) described a type of fasciation that might be considered as a tendency of the species to susceptibility to morphogenic disturbances, but there seem to be no references in the literature to the phenomenon of forking. Witches'-brooms do not appear to have been reported as occurring on red pine, although the writer observed one near the top of a tree in a 22-year-old planting.

SYMPTOMS

The first apparent symptom of forking is the extra-seasonal growth of lateral buds in the winter-bud cluster of the terminal shoot of the tree (Fig. 1, B). Extra-seasonal growth becomes noticeable in late June or

[1] A dissertation presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[3] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

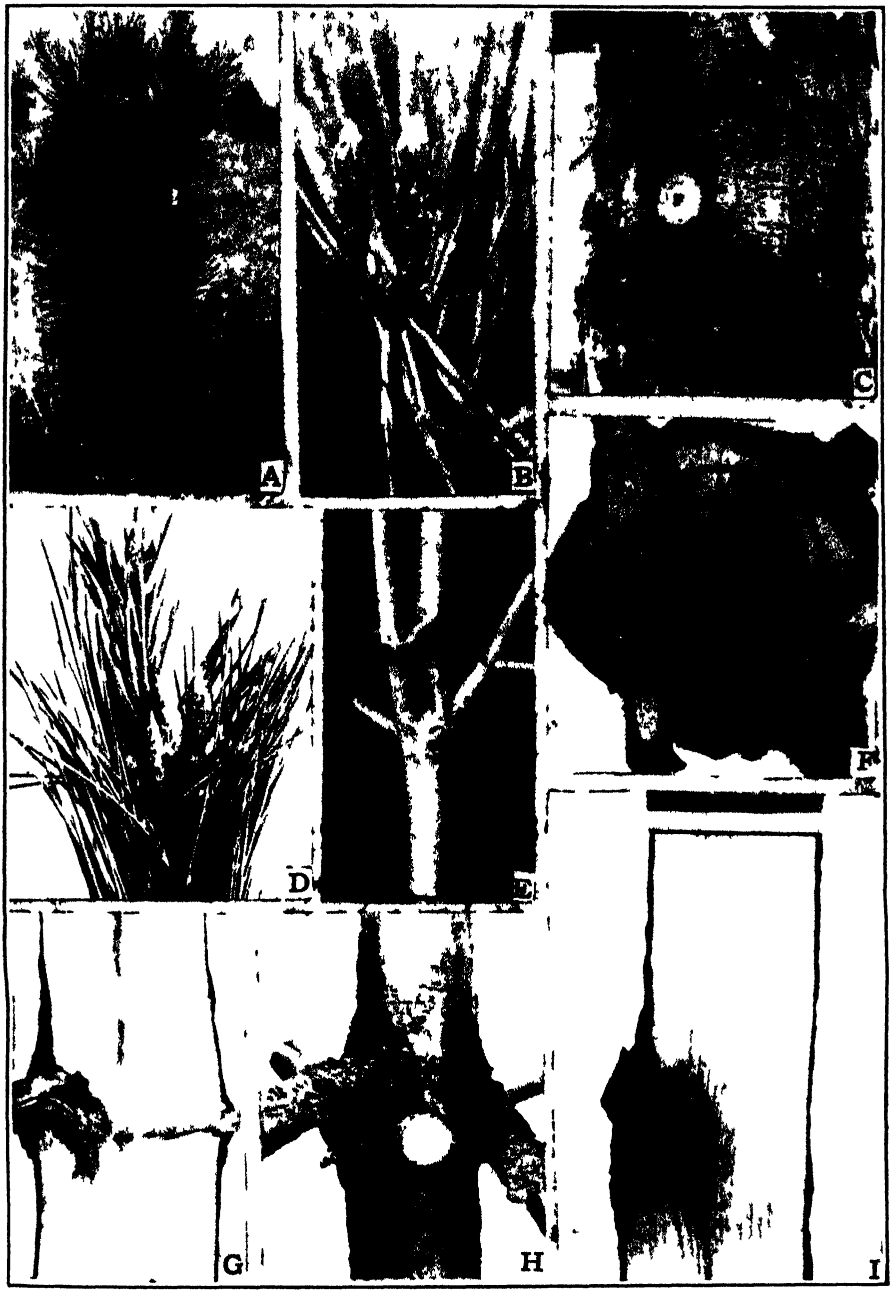

FIG 1. A. Typical fork in 7 year old red pine. B Extraseasonal growth of lateral buds taken July 5 near Springwater, N Y C Pruned forked branch on a 22 year old tree with depression immediately above D Precocious shoot rising from bud cluster with short needles and two buds at apex E Peeled fork showing secondary branching. F Cross section near a fork upon which secondary branching had occurred G Radial section of trunk showing effect of ice breakage upon a forked branch H. Effect of weight of ice upon two forked branches near top of 22 year old tree I Radial section showing characteristic appearance of a fork, with reddish brown stain extending into the wood

July, when 1 or 2, and in some cases as many as 4 lateral buds commence to grow. The resulting precocious shoots may attain a length of 3 to 4 inches, with 1 or more winter buds at their apices, before becoming dormant (Fig. 1, D). Needles develop on such shoots, but seldom attain more than half of their normal length and fail to elongate further at the initiation of growth the following spring. The precocious shoots may continue to elongate until quite late in the current season, although, according to Gustafson (5), elongation in pine shoots has been completed by early July in Michigan. Measurements of 10 precocious shoots in a planting of 8-year-old red pine were taken on August 15, 1937, and again on October 25. All but one of them showed an increase in length from ¼ to 1¾ in. The formation of a bud cluter at the tip of the precocious shoot (Fig. 1, D) interfered with normal subsequent growth, as it caused secondary branches to be formed within a few inches of the bole (Fig. 1, E).

The terminal bud of the cluster in which the extra-seasonal growth occurs seldom takes part in the abnormal development, and usually elongates at the normal time the following season. The laterals, however, during the course of their extra-seasonal growth, react as if the terminal bud had been injured or removed and grow in a more or less vertical direction. Thus the shoot, originating from the terminal bud the following season, grows in competition with one or more laterals that had commenced growth the preceding summer. This results in a bi- or trifurcated appearance of the tree, which will be referred to as "forking" (Fig. 1, A). This condition does not remain conspicuous for more than 2 or 3 seasons in most cases. Either the true leader or one of the precocious laterals will assume dominance and the other member, or members, of the fork will tend to the more or less horizontal position normally characteristic of red pine branches.

Forking may occur repeatedly in a tree. One 22-year-old specimen, when dissected, showed this phenomenon in at least 12 nodes (Fig. 2). An examination of nodes where forking had occurred at some previous time frequently showed a slight depression in the trunk immediately above the insertion of the branch (Fig. 1, C).

There seems to be no relation between the age of a tree and its susceptibility to forking, in stands up to 25 years of age. No data have been obtained upon older trees because of the difficulty of making certain, without felling the tree, that a fork might have been induced by insect or mechanical injury.

DAMAGE RESULTING FROM FORKING

The structural injury that results from forking usually has its inception during the season following the formation of the fork, and is caused by the failure of the forked members to completely unite as they increase in diameter. Because of the acute angle at which they stand in relation to each other, they are unable to slough off, or push aside, the bark that lies on the inside of the fork between their cambiums, in the manner that the growth of the trunk displaces the bark of a normal lateral branch. This

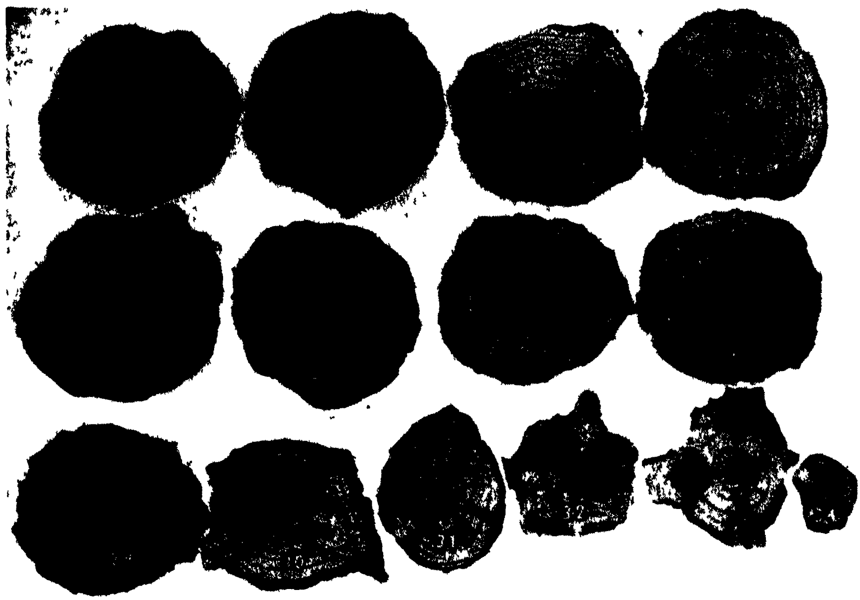

FIG. 2. A series of sections taken from a 23 year-old red pine in consecutive years from 1921 to 1934 inclusive, to show occurrence of forking and discoloration surrounding the pith.

area of non-union of cambium remains in the main axis of the tree as a fissure or pocket that becomes filled with a heterogeneous material composed largely of resin and fragments of the bark (Figs. 1, F and 1, I). These fissures are in a more or less vertical plane for several years until one of the forked members assumes dominance, as described above. While in this vertical position they are easily penetrated by water, and a favorable environment is thus established for the development of many species of parasitic and saprophytic bacteria and fungi. The presence of this flora may be an additional factor in causing the fissure to remain open.

Although it is not within the province of this investigation to evaluate the damage caused by forking from the point of view of the defects in the timber of forked trees, mention should be made of the increase of winter damage in forked stands. Since part of a forked branch may not be united organically with the trunk, the mechanical resistance to stress caused by weight of snow or ice is reduced. A branch that breaks in such a manner (Fig. 1, G) produces a more serious injury to the tree than does the breaking of a sound branch, as it lays open a cavity that may extend almost to the pith (Fig. 1, H). Normal branches are able to stand much greater stress and, if breaking occurs, usually snap off at a point beyond the trunk.

FORKING IN RELATION TO ENVIRONMENT

There appears to be no marked correlation between forking and site conditions, except a possible relationship with the mean seasonal temperature

of the locality. Determinations of soil pH in plots of forking red pine showed a range from 4.5 to 7.5 without appreciable difference in the percentage of forked trees. The disturbance occurred on soils varying from sandy types in parts of the Adirondacks to the clayey loam upon which the pine had been planted in Montgomery County, Pennsylvania. The most prevalent soil types on the Rochester Watershed were Volusia loam and Volusia shale loam. Degree and aspect of slope likewise seemed to be of no significance. There was no relation between forking and extremes of precipitation, or years of abnormal temperatures. The lowest percentages of forking encountered were in natural stands and plantations of red pine near Mt. Discovery, Essex County, New York. This locality was the most northern and the highest in altitude of those studied, which suggested that temperature and latitude might be possible limiting factors of the disturbance. Numerous observations of comparable sites, however, are necessary before any positive statement can be made on this point. Obviously, a shorter growing season would tend to minimize the effects of extra-seasonal growth, even though it might have no effect upon the primary cause.

FUNGI FOUND ASSOCIATED WITH FORKING

Isolations

Cultures were made from red pine wood immediately after sawing. The surface of the wood was sterilized by swabbing with alcohol and flaming an area of several square inches in the vicinity of the place from which the sample was to be taken. A small piece of wood in the desired locality was then excised with a sterile woodcarving gouge, flamed briefly, and thrust into a tube of 2 per cent Fleischman's Diamalt Agar.

Cultures from buds were made by dipping the bud in 70 per cent alcohol and flaming it until the alcohol was burned off and the bud scales were partly charred. The bud was then split longitudinally with a sterile scalpel and the freshly cut surfaces were placed in contact with an agar slant.

Blue-green molds commonly occurred in cultures from discolored wood from the interior of a bole in the vicinity of a fork. The majority of these are believed to be inhabitants of the wood, and not the result of poor laboratory technic, for they usually did not appear for ten days or more following inoculation of the tube of medium with the wood sample. Bacteria were occasionally present, but no attempt was made to examine them in detail, although it was ascertained that no given form was consistently isolated. While only a limited number of trees above 20 years of age could be removed from the plantings for detailed examination, some indication of the internal condition of about 30 forked trees was obtained by means of an increment borer. Frequently, it was impossible to remove a complete core with the borer because of wood rot in the vicinity of old forks about waist high on the tree. Forked areas were almost invariably accompanied by reddish brown streaks within the wood (Figs. 1, I and 2) from which fungi could be isolated. These streaks often were found surrounding the pith in forked trees.

A species of *Tympanis,* closely resembling *T. pinastri,* was cultured in several cases from discolored wood within the trunk adjacent to fissures caused by forking, and also from the interior of forked branches near their origin in the interior of the trunk. In one instance this species was cultured from the wood of a 7-year-old tree that was, apparently, in a perfect state of health, except for a fork that had occurred 2 years earlier.

Comparison of the species of *Tympanis* found in this investigation with cultures of *Tympanis* sp., kindly furnished by Dr. J. R. Hansbrough, indicated that the organism was the same species that was involved in his studies of the Tympanis canker of red pine (7, 8).

There is considerable evidence that *Tympanis* enters the tree, in the majority of cases, through fissures resulting from forking. The writer also believes that the depressed areas on the trunk above the insertion of a branch may, in many instances, result from the internal fissure caused by forking. As such depressions may, according to Hansbrough, be a symptom of *Tympanis*, a careful study of the internal anatomy, in addition to cultural investigations, seems advisable before making a diagnosis in the absence of fungus-fruiting bodies. Depressions accompanied forked branches in the majority of cases studied, in which the node was 10 or more years of age. Observations based upon the external appearance of depressions of 118 trees showed that the branch at the base of the depression had been a member of a fork in at least 84 per cent of the cases. The true figure probably would approach 100 per cent, since previous forking could not always be determined from external observations on account of the size and age of the nodes near the base of large trees and close pruning of lower branches in the stand.

Association of Dematium pullulans with Forking

The organism most consistently cultured from forked red pine was identified by Dr. David Linder of the Farlow Herbarium as a variety of *Dematium pullulans* de Bary.

Dematium pullulans was cultured from the lateral and terminal buds, pith, discolored wood surrounding the pith, and discolored wood in the root crown of red pine from the Rochester Watershed. It was also cultured from buds of forked red pine growing in southeastern Pennsylvania. It was the only organism to develop in significant percentages from red pine buds. Approximately 75 buds were used as material for culture, and 64 per cent of the resulting organisms proved to be *D. pullulans.* It also was found occasionally in cultures from other parts of the tree: twice in forked branch traces, twice in the pith, and once in the root crown. Cultures from the roots failed to show *D. pullulans,* although it is possible that it was not noticed because of the vigorous growth of bacteria and blue-green molds in some of the cultures. Although positive identification of the fungus in the tissues could not be made by means of an histological study, the presence of a catenate type of hyphae in the pith (Fig. 4, F) strongly indicated that the fungus under observation was *D. pullulans.* The probability of the

accuracy of this determination was strengthened by the lack of any cultural evidence of the presence of fungi possessing a mycelium of this type other than *D. pullulans.* Resistance of the hyphal wall to Cartwright's stain (1), which characterizes the chromatic hyphae of *D. pullulans,* was also typical of the pith-inhabiting organism.

Several members of the *Dematiaceae* have been recognized for some time as wood-inhabiting organisms. Siggers (13) refers to *Torula ligniperda* as a cause of structural weakness and brown staining in tulip poplar and white ash. Reddish-brown staining was usually present in the vicinity of old forks in red pine wood and was of frequent occurrence surrounding the pith in trees affected by the disturbance (Fig. 2).

Dematium pullulans is readily grown upon a wide variety of culture media, both liquid and solid. Cultures in flasks on sterile red pine sawdust, moistened with distilled water, produced a mycelium and conidia, but the yeasty conidial masses, characteristic of its growth on other media, were not evident. A series of cultures was made by placing a block of agar 5 mm. square, containing the fungus, upon the surface of steam-sterilized soil in large culture tubes. Growth took place readily and the hyphae penetrated the soil to the bottom of the tube: a depth of about 6 cm. Single-spore cultures were made by means of the Kienholz technic (11), and the following observations were made on sub-cultures of these on prune agar and on liquid 2 per cent malt.

MORPHOLOGICAL OBSERVATIONS UPON DEMATIUM PULLULANS

Dematium pullulans de Bary: The mature conidia are typically ovoid to ellipsoidal, averaging $7.4 \times 4.0\ \mu$, 2-guttulate and nearly hyaline. Variations from this type occasionally occur (Fig. 4, A), especially in liquid culture. In hanging-drop cultures the spores may reproduce by budding or germinate directly, forming hyphae upon which conidia may be borne laterally (Fig. 4, E). Freshly transferred cultures on prune agar produce conidia so abundantly that a yeasty mass, varying from white to light violet, is formed upon the surface of the culture. The vegetative mycelium is usually somewhat submerged and black in macroscopic appearance, forming a compact stroma-like layer at the surface. The mycelium varies from a dark *Torula*-like form, frequently found in liquid cultures, to a hyaline, distantly-septate type. It may contain granular material or drops of oil in some instances (Fig. 4, E). Round, black bodies, usually less than 1 mm. in diameter, often appear on the surface of prune-agar cultures 2 weeks or more old. These structures are composed of a black, highly-compacted outer layer with a pseudo-parenchymatous, readily stainable central core. Such bodies will be referred to as microsclerotia. Dark-ringed tapering hyphae often appear in tufts on the surface of microsclerotia, although they may also arise directly from the mycelial surface. Bulbilous structures, apparently of similar nature to the rings, frequently occur at the base of a hyphal branch. A type of exospore, resembling spores of the genus *Coniothecium,*

was found among these hyphae and was abundant also in liquid cultures. These spores were ovate to orbicular and usually very dark. They averaged 24 μ in length, and often had one or more hyphal cells attached to their distal end.

A graded pH series was set up with Elliott's medium (3), which contains asparagin as a nitrogen source; and a second series was run with 2 per cent malt. The agar was omitted from these media and the pH was adjusted with HCl and NaOH. The malt series of 12 pH values from 1.8 to 8.5 showed growth from 1.8 to 7.2, although growth was slow at the extremes of the range, particularly at the acid end. Optimum growth occurred between 5.5 and 6.5. On Elliott's medium at pH values from 2.2 to 8.4, growth was obtained from 2.2 to 7.2, with an optimum range betweeen 5.0 and 6.5. The malt series was inoculated with mycelium, while conidia were used with Elliott's medium.

Growth of different cultures of the fungus collected from various localities showed no tendency toward morphological variation when grown upon 2 per cent malt agar in Petri dishes. Numerous varieties of *Dematium pullulans* have been described, but the variety with which we are here concerned cannot be placed satisfactorily among these. In the character of its nodose hyphae it resembles var. *fimicola* Marchal, but differs markedly from it in spore characters.

EXTENT OF FORKING IN STANDS

A partial summary of data obtained from some of the plots studied on the Rochester Watershed is given in table 1. It is evident from data upon plots C and D that the more vigorous trees are the most susceptible to forking. This seemed to apply to trees in all of the age groups studied, although quantitative data are not available for trees of the older classes.

TABLE 1.—*Amount of forking with related data on sample plots*[a] *on the Rochester watershed*[b]

Plot	Trees examined	Age	Trees forked	Trees with precocious buds in terminal 1937 cluster	Trees with secondary lateral near main stem	Average height of stand	Average height of forked trees
	No.	*Years*	*Per cent*	*Per cent*	*Per cent*	*In.*	*In.*
A	417	10–11	92			121	
B	100	7–8	63				
C	404	6–8	69			27.1	30.0
D	100	5–7	78	30	40	33.1	34.5
E	60	5–7	88	10	38	37.1	37.1
F	88	6–10	92	30	51		
G	100	23–25	94		22	324	.
H	100	7–9	87	41	57		.

[a] All plots represent different plantings except E and F which are from the same lot.
[b] Blanks in the table indicate that no data were taken.

A map was made of two plots (C and G, Table 1) to determine whether foci of infection occurred in forked stands. The map indicated that forking was evenly distributed throughout the stand, as no evidence of "islands" was found. Two stands of natural red pine within 30 miles of the watershed showed percentages of forking that fell in the range of those of the plantings. In most cases the plantings were of pure red pine, although in some of them there was a light admixture of Norway spruce and eastern white pine, which apparently had no effect upon the prevalence of forking.

The disturbance seems to reach a maximum after a period of years and then gradually subside, although there are indications that there may be a second cycle in the same planting. Annual occurrence of forking when plotted on coordinates (Fig. 3) may be considered additional evidence that an organism was the causal agent of the disturbance.

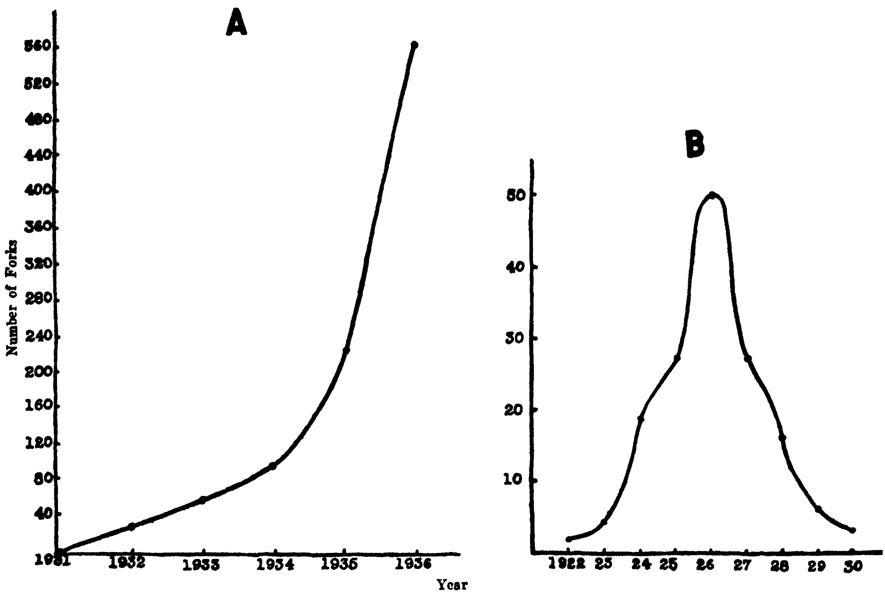

FIG. 3. Annual occurrence of new forks in plantings of red pine. A. Distribution of forks on 852 trees, 6–7 years old, from five plantations. B. Distribution of forks on 100 trees from a plantation in which the trees averaged 23 years old.

HISTOLOGICAL STUDIES

Material for histological examination was fixed in a chromic acid—formalin—salcylic acid solution (2), dehydrated with the N-butyl alcohol technic, and cut on a sliding microtome in sections varying from 5 to 15 μ in thickness. It was found necessary in working with the older tissues to extend the period of dehydration and infiltration to 10 days or 2 weeks in order to insure complete penetration by the alcohol and paraffin. Buds, precocious shoots, wood from the older portions of the trunk, and roots were examined for abnormal anatomical features and for the presence of fungus hyphae. The most successful stain employed was Cartwright's picro-analine blue combination (1), although the chromatic hyphae that appeared in the old wood

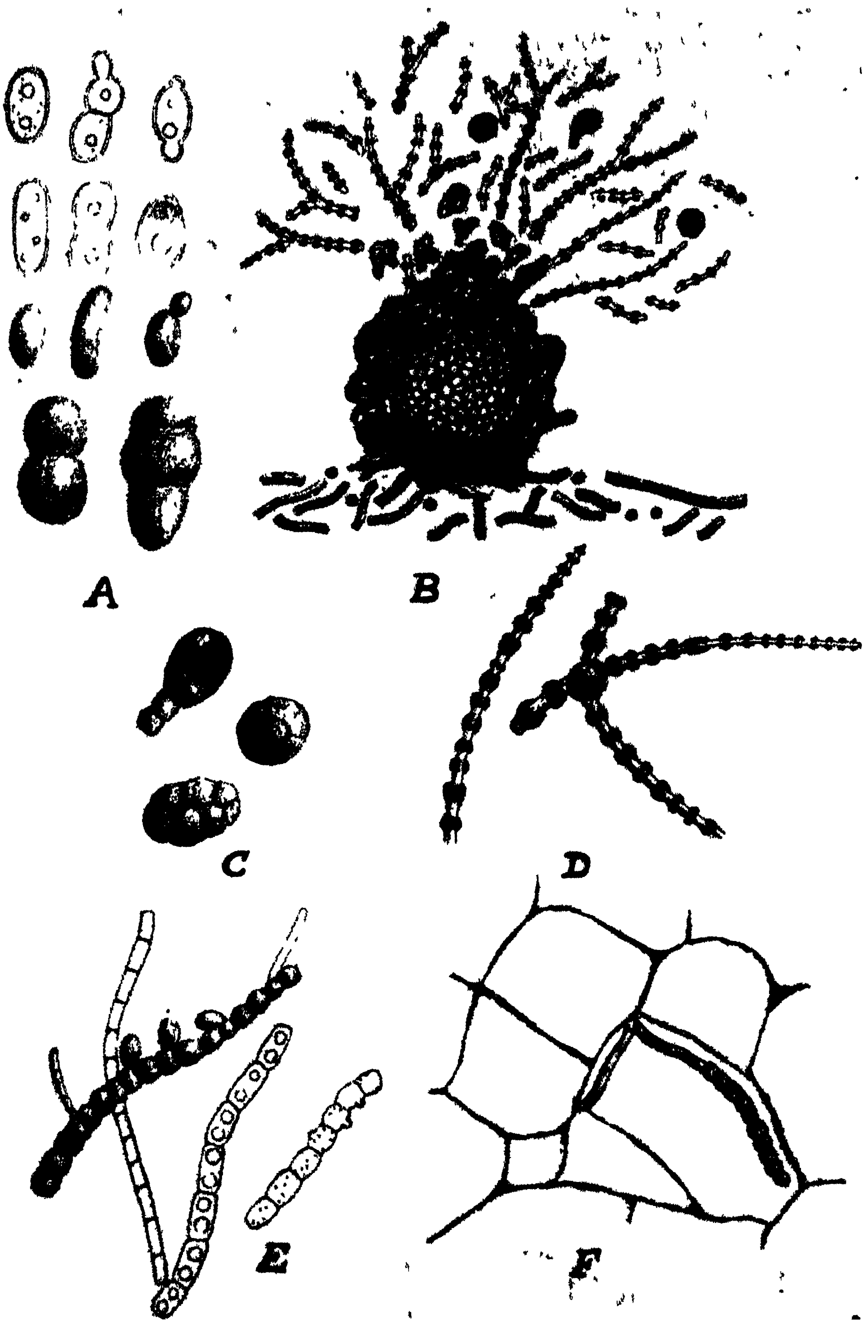

FIG. 4. *Dematium pullulans* de Bary. A. Types of conidia; the spore in the upper left is the typical form. B. Section through a microsclerotium with nodose hyphae and *Coniothecium* like spores. C. *Coniothecium* like spores as found among nodose hyphae and in liquid culture. D. Nodose hyphae. E. Types of hyphae. F. Hyphae, believed to be of *D. pullulans* in pith cells of *Pinus resinosa* (drawing from camera lucida).

types of varying hydrogen-ion concentrations upon which forking occurs. Gustafson (6) found that red pine, brought into the greenhouse in January, would commence growth at once and form 2 bud clusters by July. Apparently the terminal bud develops normally in this case, and there would seem to be no connection with the type of growth that results in forking.

It cannot be proved conclusively that a genetic factor might not be causally connected with forking, but several circumstances are contrary to such a possibility. The wide geographic range of the occurrence of forking, together with the fact that forking in neighboring stands may gradually attain a maximum in different years and at different ages of the plantings (Fig. 3), indicates genetic influence to be unlikely.

The presence of fungi in the buds and internodal pith, as indicated by the results of cultural and histological studies, forms the basis of the writer's suggestion of a theory of the fungus origin of forking. Research on phytohormones has proved that bud inhibition and activation are regulated, at least in some instances, by auxin. It also is well established that certain fungi produce auxin in considerable quantities. Thus, the extra-seasonal growth of laterals might be attributed to the production of auxin within the bud by fungus hyphae. Le Fanu (12) showed that in two shoots of *Pisum*, one of which was being strongly inhibited by the other, the dominant shoot indicated a high concentration of auxin with the *Avena* test, while the inhibited shoot showed only slight traces. If a similar condition occurs in the bud clusters of pine, it would follow that the presence of a phytohormone produced by a fungus in the lateral buds might conceivably act as a stimulus to extra-seasonal growth. Production of the hormone in the terminal bud would not necessarily lead to extra-seasonal stimulation if the hormone were normally present in considerable quantities. The abundant production of growth substance by *Dematium pullulans* and the proof of its ability to inhabit red pine might be regarded as indicative of the possibility that the fungus could be causally connected with the disturbance. It is impossible to compare the microflora of normal trees with those of this investigation. Since forking may be latent in a tree, it could not be determined with certainty that a given specimen was completely normal.

SUMMARY

An abnormal bifurcation of red pine trees is prevalent in New York State and other sections of the northeastern United States. This condition is caused by extra-seasonal shoot elongation in which the lateral buds take part while the terminal remains in the dormant condition. Failure of the inner surfaces of the resulting fork to unite completely during subsequent growth of the tree causes an open fissure in which wood-destroying fungi may enter. *Tympanis* sp. also frequently gains entrance in this manner. No correlation was found between rate of forking and site conditions. Trees at high altitudes in the northern part of the area studied showed the lowest percentage of forking. *Dematium pullulans* de Bary was isolated from

abnormal buds in the majority of cases, and hyphae of the characteristic form of the organism were found in the wood of abnormal trees. Young red pines were inoculated hypodermically with conidial suspensions of *D. pullulans* and, after 5 weeks, the fungus was reisolated from sections of the stem 1 and 2 cm. from the site of inoculation. *D. pullulans* was found to contain a growth promoting substance by means of the lupine technic in a quantity exceeding that of other fungi tested. The theory is advanced that abnormal growth of a pine bud may be initiated through the action of a phytohormone produced by the fungus living within the tissues.

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CORN-SEEDLING VIRESCENCE CAUSED BY ASPERGILLUS FLAVUS AND A. TAMARII

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A color reaction of corn seedlings known as virescence, heretofore unreservedly attributed to genetic factors, has been fully reproduced by the writers by fungus action on corn kernels that do not carry the *vv* factors. Apparently, the products of metabolism of these fungi or their interactions with the host plant affect the seedlings in an abnormal way in exactly the same manner as do certain genes and may serve as a clue to the manner in which certain genes operate.

After considerable experimentation and numerous successes and failures at reproducing this disease, the writers now feel that the governing factors

are sufficiently understood, empirically, so that the facts warrant publication. Much of the fundamental nature of this disease, which may be called "induced virescence," remains to be learned.

SYMPTOMS OF INDUCED VIRESCENCE

Strongly virescent-reacting plants, resulting from inoculation with the causal organisms, can be detected when the coleoptile projects only 2 cm. from the ground and before the first leaf has emerged. In the 3-leaf stage (Figs. 1, 2) the oldest (lowest) leaf frequently showed the most greening,

FIG. 1. Corn-seedling virescence induced by *Aspergillus flavus*. A. Normal seedling. B. A common type of virescence, greening starting at the tips of the leaves and in the coleoptile. C, D. Green color developing as stripes along the principal veins.

but this was not always true. The extent of chlorophyll deficiency may range all the way from only traces of green in the leaf tips through intermediate stages up to wholly green plants. The areas lacking chlorophyll vary in color from almost white to cream or pale green.

Greening proceeds especially from the tip downward, but it frequently also develops at the same time from the base upwards. The greening may proceed gradually without streaking or it may appear definitely as streaks along the principal veins (Fig. 1). The greening from the base, when it occurs, often proceeds especially along and outward from the midrib. If chlorophyll is very deficient, the plants may die in the 3-leaf stage. If, on the other hand, there is sufficient chlorophyll for continued growth, the roots at the crown develop, and all succeeding leaves after the third or fourth are fully green, although growth in size may, for a while, almost cease (Fig. 3).

Only rarely, and under the most favorable conditions for the expression of virescence, did all the plants in a pot planted with inoculated open-pollinated seed exhibit chlorophyll deficiency. Usually, the reaction was

as is shown in figure 2, where some plants are almost white, some partly green, and a considerable number of them normally green. Temperature, light, soil conditions, extent of seed-coat injury, and concentration of spore suspension, all, had an effect on the extent to which virescence was expressed.

Final notes in greenhouse experiments were made when most of the plants were in the 3-leaf stage. Some greening had taken place by that time, but not enough to obliterate symptoms that showed earlier; and all weak plants that were able to emerge had done so by that time.

FIG. 2. A population of a variety of open pollinated dent corn, some of the seedlings being much more resistant to "induced virescence" than others. Similar segregation for resistance and susceptibility occurred in all open-pollinated varieties tested.

Plantings made in field plots showed the same kind of variation shown in figure 2. Plants that were half or more green by the time they reached the 3-leaf stage survived for the most part. Some of these were extremely weak (Fig. 3), while others produced more or less grain at maturity.

GENETIC VIRESCENCE COMPARED WITH INDUCED VIRESCENCE

In the course of corn-breeding studies at the Illinois Station, many inbred ears are tested on greenhouse benches. In the seedlings thus produced the writers believe they have seen genetic virescence of every type of appearance coinciding with the symptoms of virescence induced by *Aspergillus*. Twenty types of genetic virescence have been numbered by various investigators (1, 2, 3, 4, 9, 10) and a few additional ones have been studied. Although some of these differ considerably in appearance, many are phenotypically

FIG 3 A. Young corn plants, both grown from kernels from which the seed coat had been removed from the crown before inoculation with *Aspergillus flavus*. One plant failed to become virescent, while the other developed only enough green to barely survive through the seedling stage, and was in the 6 leaf stage and fully green when photographed. B. The same 2 plants at maturity photographed from approximately twice the distance The weak plant produced a small tassel and ear shoot but no grain.

either similar or alike and can be distinguished only by their linkage relationships. Only in one case, V_{17}, was greening described as being more pronounced at the base of the leaf than at the tip. In all other cases, where mention of the position is made, greening is mentioned only as beginning at the tip. Lindstrom (9, Plate 1), however, illustrates slight green color at both the tips and bases of nearly white leaves. In none of the brief descriptions of genetic virescence is green striping along the veins, as shown in figure 1, mentioned. This occurred frequently in induced virescence and was also observed by the writers in some types of genetic virescence.

Most writers describing genetic virescence observed that the chlorophyll-deficient plants were smaller than the normal ones in the seedling stage. In measurements of 4460 plants, those exhibiting induced virescence average 1.9 cm. shorter than those similarly treated, but green in color (Table 1).

TABLE 1.—*Influence of seed-coat injury and seed inoculation with spores of Aspergillus flavus on plant height, blighted plants, visible rots, and internal infection in mesocotyl of corn seedlings. Data taken when most plants were in the 3-leaf stage*

Plant classification	Height of plants	Plants blighted whole or in part	Mesocotyl infection		
			Visibly rotted	Total internal infection[a]	Internal infection with *A. flavus*
	Cm.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Noninoculated control plants					
Seed coat sound	18.7	0.2	5.3	18.8	0.6
Seed coat removed at crown	11.8	0.7	9.7	37.5	0.6
Seed inoculated with *A. flavus*					
Seed coat sound	18.4	0.7	4.2	31.7	18.8
Seed coat removed at crown, plants green	11.3	1.3	18.0	50.0	31.3
Seed coat removed at crown, plants virescent	9.4	5.2	19.5	58.2	48.6

[a] Total infection with various organisms—Penicillia, Fusaria, and Mucors, being prominent in addition to *A. flavus*. Bacteria were largely eliminated by using acidified medium.

In working with "yellowish virescent," Carver (1) found that under field conditions about one-third of the plants matured. These were about half as tall as normal plants and produced some pollen but no ears. This compares very well with the condition illustrated in figure 3. Mature plants from some other types of genetically virescent seedlings produced small and inferior ears (10).

FACTORS INFLUENCING VIRESCENT REACTION

SEED-COAT INJURY

A break in or removal of part of the seed coat of corn kernels before inoculating with spores of *Aspergillus flavus* or *A. tamarii* and planting is a prerequisite to readily incident virescence. Even without artificially injuring the seed coats, if other conditions are favorable, an appreciable number of virescent seedlings usually develop from inoculated seed of corn types with rough indentation, because of seed coat breakage at the crown from normal handling.

Experiments were conducted with various kinds of artificially made seed-coat injuries differing in location and extensiveness. The appearance of these injured kernels was illustrated in another publication (6, Fig. 10). Certain types of seed-coat injury, without seed inoculation, but incident upon simply planting the grain in corn-belt soils, resulted in considerable pathological disturbance to the plants grown therefrom (8).

When the entire seed coat was removed from the crown and the kernels were then inoculated, 69.7 per cent produced virescent plants. Removal of one-fourth the seed coat from the crown caused 44.5 per cent, and a cut of about 1/16 inch across the crown without removing any seed coat caused 20.0 per cent. A cut through the seed coat into the side of kernel and slightly into the horny endosperm, but not exposing soft endosperm, caused 43.5 per cent virescent plants. Breaking off the tip cap from the kernels caused 14.1 per cent, and there were 1.2 per cent virescent plants in the checks with apparently sound seed coats, which also had been inoculated. These are averages from several tests conducted in the winter in a greenhouse and involved approximately 500 crown-injured kernels and the same number of uninjured checks. The station strain of Reid Yellow Dent open-pollinated corn was used in this and all other tests herein reported, except where other types are specifically mentioned.

CORN VARIETIES, INBREDS, AND HYBRIDS

Of 20 varieties and hybrids tested, all produced induced virescence prominently. These included 7 hybrids of dent corn, 4 open-pollinated varieties of dent corn, 3 of flint corn, 3 of sweet corn, 2 of popcorn, and 1 of flour corn. One hybrid was outstanding in producing 100 per cent virescent plants in several repeated tests. The flour corn and the closely selected dent variety known as "Illinois high protein" averaged about 90 per cent virescent plants in the same tests. All of the remainder were somewhat lower but none of them outstandingly low. Only in certain inbreds (5, Fig. 19) was a high degree of resistance to induced virescence found.

LIGHT

That light has an influence on the virescent reaction was demonstrated in 2 tests. Ten 1-gallon jars were each planted with 20 kernels of crown-injured seed inoculated with *Aspergillus flavus*, 10 were inoculated with *A. tamarii*, and an additional 5 were planted with sound noninoculated seed to check on the possibility of the seed carrying factors for genetic virescence. These were placed intermingled on a greenhouse bench, where they received daylight only. A duplicate set received continuous light. When the sun was not shining, 500-watt Mazda bulbs in reflectors placed 3 feet above the plants were used. The greenhouse temperature was 20–22° C.

The first test was conducted during January. Under daylight only, *Aspergillus flavus* caused 50.6 per cent virescent plants and *A. tamarii* caused 38.4. Under continuous light the percentages were respectively 25.8 and 15.5. The second test was made during March. This time, under daylight only, *A. flavus* caused 18.4 per cent virescent plants, while *A. tamarii* caused 16.4. Under continuous light the respective percentages were 10.8 and 6.2. The checks in all cases remained almost free from virescence.

The second test was grown under longer daylight than the first and also produced considerably fewer virescent plants. That the reduction in vires-

cence in the second test, as compared with the first, was due to difference in light alone cannot be stated for certain. There appears to be no doubt, however, that within each individual test conducted at the same time the differences in virescence were caused by differences in amount of light.

TEMPERATURE

Temperature also, is of importance in influencing the degree of virescent expression following inoculation. Experiments in which Wisconsin-type soil-temperature tanks were used were conducted in the spring of 2 different years. In 1936, *Aspergillus flavus* inoculations at a soil temperature of 16° C. produced 56.6 per cent virescent plants. At a soil temperature of 30° C., only 3.2 per cent occurred. In 1937 similar inoculations at 18° C. soil temperature produced 28.6 per cent virescent plants, while, at 30° C. soil temperature, only 1.3 per cent occurred. In all cases the air temperature varied within about 2° of the soil temperatures. Inoculations with *A. tamarii* also were similarly affected by temperature, but not to so marked an extent.

SOIL MOISTURE

Soil moisture is another important factor influencing the development of induced virescence. Experiments were conducted in the greenhouse with different batches of dark silt-loam soil in the early spring of 3 different years. The results were very similar, and only a summary need be given. The growth of corn in dry soil with only about 18 per cent moisture, dry basis, was compared with wet soil of about 27 per cent moisture. The latter soil could still be screened and handled satisfactorily without puddling, and emergence of corn seedlings was 3 to 4 days earlier than in the drier soil.

Inoculation of crown-injured corn kernels with *Aspergillus flavus* produced an average of 14.3 per cent virescent plants in the dry soil, and 51.0 per cent in the wet soil. *A. tamarii* produced, respectively, 7.5 and 48.6 per cent. Not only was there a decided numerical difference in virescent plants with respect to soil moisture, but chlorophyll deficiency per virescent plant was more pronounced in the damper soil. When considering only the plants that were more than half white, there were 1.6 per cent in the dry soil and 23.7 per cent in the damp soil. Adding more water immediately after planting, to bring the calculated soil moisture up to 35 per cent, had no additional effect on increasing virescence.

PREVIOUS SOIL CROPPING

In order to circumvent one of the uncertainties of securing virescent development, it was found necessary to use soils that had been stored 3 or more months without cropping. Whether the soil was kept damp or gradually allowed to dry in storage appeared to be immaterial. The soil was held at storage temperatures of 60° F. or warmer.

Soils brought from cropped land in the fall usually produced a less satisfactory virescence than did those that had been stored in a basement

from the previous year. Soil taken from different locations, however, varied considerably with respect to this reaction; but this difference disappeared after the soil had been in storage for some months. Soils taken from different cornfields in the fall gave just as much variation in results as did those that had been cropped with other plants.

While corn seedlings usually gave a good virescent reaction when grown on soils that had not been cropped for some months, yet, when the same soil was used for repeated tests in the greenhouse, it frequently gave poor virescence or nearly failed altogether in the third successive test. This was true regardless of whether Aspergillus inoculations had or had not been made in the previous tests. Sometimes, the previous cropping of soil with soybeans in the greenhouse also reduced virescence. In a test in late spring, when the temperature was too high for best virescent reaction, stored soil produced 30 per cent virescent plants, while cropped soils, different lots of which had just produced in the greenhouse mature crops of alfalfa, red clover, timothy, brome grass, and bluegrass, produced no virescent corn plants at all.

Attempts to improve the virescent reaction of corn on soils that were in a poorly reacting condition, by the addition of nitrates or complete fertilizer, or by sterilization with heat or formaldehyde, failed. Soil held in storage for several months, however, never failed. Attempts to decrease the virescent reaction of corn on stored soil by the liberal addition of chopped straw, well mixed with the soil a week before planting the inoculated corn, also was ineffective.

No satisfactory clue has yet been found as to the nature of the soil factors that have a marked influence on the development of induced virescence in corn seedlings.

SOIL TYPES

Dark silt loams, clay, fine gravel, greenhouse compost, blow sand and pure silica sand were all used successfully in producing induced virescence in corn seedlings grown from Aspergillus-inoculated seed. Little difference was noted, except that the chlorophyll-lacking areas appeared to be slightly more extensive in seedlings grown in soils rich in nitrates.

CAUSAL ORGANISMS

Aspergillus flavus and *A. tamarii* were found to cause induced virescence of corn seedlings under proper conditions. Ten different isolations of the former and 2 of the latter, isolated from several sources at different times, were used in the tests. All of them caused induced virescence, although some were slightly more effective than others. Two of the *A. flavus* isolations decidedly retarded the growth of the seedlings, but this gave them no advantage in the virescent reaction.

The following fungi, also used in inoculation tests on crown-injured corn, failed to cause induced virescence: *Aspergillus wentii, A. ochraceus, A. glaucus* (2 strains), *A. versicolor, A. niger, Penicillium notatum, P. palitans,*

P. viridicatum, P. expansum, P. oxalicum, Fusarium moniliforme (6 strains), *Diplodia zeae, Gibberella zeae*, and *Trichoderma* sp.

SPORE LOAD

Inoculations usually were made by soaking the kernels with broken seed coats for 1 or 2 minutes in spore suspensions containing about 10^7 spores per cc. Other spore concentrations with *Aspergillus flavus* also were tried. In one test spore concentrations of 10^7, 10^6, 10^5, and 10^4, produced, respectively, 37.3, 26.6, 26.7, and 24.8 per cent virescent plants. In another test, concentrations of 10^7, 10^5, and 10^3 produced, respectively, 69.7, 48.5, and 4.1 per cent.

ANTAGONISM OR COMPETITION

A number of fungi were used for inoculation simultaneously with *Aspergillus flavus* to test for possible effects from antagonism or competition. Inoculations were made in two concentrations; *A. flavus* spore suspensions of 10^7 and 10^5 spores, respectively, per cc. were mixed with an equal volume of 10^7 spores per cc. of the other fungus. Thus, in the latter case, there were approximately only 1/100 as many spores of *A. flavus* as of the other fungus. Adding *A. wentii, A. ochraceous, Penicillium palitans*, and *Trichoderma* sp. produced no appreciable effect on the virescent results when both cultures were at 10^7. *Aspergillus niger, P. viridicatum, P. notatum, P. expansum*, and *Fusarium moniliforme*, however, reduced virescence significantly under the same conditions. When *A. flavus* was used at a concentration of only 10^5, while the others were used at 10^7, all reduced virescence to half or less as compared with *A. flavus* at 10^5 alone, while *A. niger, P. viridicatum, P. expansum*, and *F. moniliforme* stopped the development of virescence entirely.

SEEDLING INVASION AND ROTS

Merely removing the seed coat from the crowns of corn kernels may allow decided pathologic effects in the consequent seedlings. In greenhouse tests with dark silt-loam soil taken from ordinary cropped fields near Urbana, the heights of seedling plants were checked and visible mesocotyl rots and internal mesocotyl infections were increased, (Table 1). The data are based on 4460 plants measured and blights observed, and 590 mesocotyls inspected and plated. When mesocotyl rots were found, they usually occurred near the lower end, adjoining the seed. Internal mesocotyl infection was determined by surface sterilizing for 1 minute with 1 : 1000 mercuric chloride and plating as previously described and illustrated (7, p. 77).

Seed treatment with suitable disinfectants largely prevented the pathologic effects that otherwise followed seed-coat injury (8).

Inoculating the seed with *Aspergillus flavus* had no noticeable outward effect on the seedlings when the seed coats were sound. A marked difference was observed, however, when the mesocotyls were plated, there being internal infection with this fungus in 18.8 per cent of the inoculated plants and only

0.6 per cent in the controls. When the seed coats had been removed from the crowns and the seeds inoculated with *A. flavus* before planting, the plants could be classified into 2 groups, *i.e.,* normally green and virescent. The normally green plants, grown from inoculated crown-injured seed, were nearly as vigorous as those grown from noninoculated crown-injured seed, but there was a significant increase in mesocotyl rots and internal mesocotyl infections in the former group. The virescent plants averaged shortest of all and had the highest percentage of mesocotyl infection.

There was nothing unusual about the mesocotyl rots being aggravated by seed inoculation with *Aspergillus flavus* or *A. tamarii,* or the occurrence of these fungi within the tissues of many apparently sound mesocotyls. Examination of young corn plants grown from seed inoculated with *A. niger, Penicillium oxalicum,* and *Trichoderma* sp. also revealed pronounced increases in mesocotyl rots and internal infections as compared with the checks. These latter fungi, however, caused no virescent reaction.

FIELD EXPERIMENTS

In field experiments conducted with crown-injured seed inoculated with *Aspergillus flavus* or *A. tamarii,* virescence developed abundantly in 3 years out of 5. In the other 2 years, virescence was rare. Coincidentally, soil moisture had been abundant prior to and for some days following planting time in the years when virescence occurred prominently, while the soil had been rather dry at planting time in the other 2 years. These results agree with those obtained in greenhouse experiments on the effect of soil moisture on the occurrence of induced virescence.

Inoculated corn, planted May 30, 1935, under excellent growing conditions following weeks of damp weather, produced 49 per cent virescent plants despite the long periods of daylight at that time of the year. The initial stands, as counted 12 days after planting, were very good: 95.8 to 92.3 per cent for the different lots. When recounted, 30 days after planting, the stand from good seed with sound seed coats had decreased 5.4 per cent, while similar seed, but with the seed coats removed from the crown, had decreased 11.8 per cent; and when the latter kind of seed had been inoculated with *Aspergillus,* the loss in stand below the original count was 18.9 per cent. A considerable part of the loss in stand in the last-named instance (where crown-injured seed had been inoculated) was caused by lack of sufficient chlorophyll for the plants to survive. Some other seedlings just managed to survive and produced very weak plants (Fig. 3). The average heights of the plants 30 days after planting were, respectively, for plants grown from kernels with sound seed coats not inoculated, injured seed coats not inoculated, and injured seed coats inoculated, 25.7, 17.5, and 14.1 inches. The variability in height within each group was the least in the first group and the greatest in the last. The acre yields from the same groups in the same year were 57.6, 39.8, and 28.8 bushels, respectively.

Dry weather conditions prevailed during the spring of the previous year (1934), there being only barely enough soil moisture for germination and growth at and for some time after planting on May 14. Virescence failed to develop from the inoculations. Field stands from the 3 groups: sound, noninoculated seed coats; injured, noninoculated seed coats; and injured seed coats inoculated with *Aspergillus flavus,* were, respectively, 89.8, 89.0, and 88.9 per cent. Acre yields from the same 3 groups, respectively, were 41.6, 35.0, and 36.2 bushels. Thus, *A. flavus* inoculations under environmental conditions where virescence did not develop, failed to influence either the field stand or the yield of grain. Merely removing the seed coat from the crown, however, did significantly increase seedling mortality and decrease the yield of grain.

DISCUSSION

Heretofore, the term virescent seedlings has been used by other investigators only for the type that is directly controlled by hereditary genes. As the same kind of color effects can be produced in corn in which these hereditary genes are absent, the writers have been using the terms "hereditary virescence" and "induced virescence," to distinguish the 2 types.

Induced virescence, however, is governed also by hereditary factors, to a certain extent. Inbred lines can be produced that are highly resistant to this reaction, following inoculation with *Aspergillus flavus* or *A. tamarii.* Other inbreds, under the same conditions, are highly susceptible to induced virescence, although they contain none of the genetic factors directly responsible for virescence. All open-pollinated lots of corn, when tested under conditions suitable for the development of induced virescence, produced mixed populations of normal and virescent plants. Environmental factors, however, greatly influenced this reaction; so that the percentage of virescent plants might vary all the way from 0 to 95 in different tests of grain from the same ears.

Environmental conditions favorable to the development of a high percentage of virescent plants were also conducive to a greater chlorophyll deficiency per virescent plant. In other words, nearly white plants occurred only under conditions very favorable to incidence of virescence.

The development of induced virescence and genetic virescence is influenced by temperature and light in the same manner. In addition, however, induced virescence is influenced also by the soil complex. This complex may or may not be favorable to the interaction between host and parasite that results in induced virescence. As this type of virescence develops when pure quartz sand without the addition of nutrients is used as the substratum, it appears that the soil conditions in which virescence does not develop are of an inhibitory nature. The factors that bring about this inhibition are not yet understood. In numerous experiments the writers have so far not failed to put soil into proper condition for the development of induced virescence by first storing it in bins or covered metal cans for some months prior to

using and keeping the soil moisture moderately high while the corn is germinating. Soils taken from fields at planting time in the spring usually were satisfactory, but those taken in the fall after being cropped all summer often inhibited the development of induced virescence. Genetic virescence was not influenced in that way.

In both genetic and induced virescence, when the plants were in the 3-leaf stage, the fully green ones averaged somewhat taller than those deficient in chlorophyll. The difference in amount of chlorophyll no doubt was responsible for this. In addition, in the Aspergillus-inoculated plants, the virescent seedlings had somewhat more internal mesocotyl infection than those resistant to the virescent reaction (Table 1). This, however, may have been affected also, by chlorophyll deficiency. There appears to be no evidence that the virescent plants were virescent because of more intensive infection by *A. flavus* as compared with the similarly inoculated plants that developed normal green color. The principal reason for the difference in color reaction seems to be an inherited resistance of the plant, not to invasion by the fungus, but to the ability of the fungus to bring about the reaction that results in virescence.

The ultimate agent responsible for the virescent condition no doubt is the same whether the virescence be hereditary or induced. This is inferred not only because both types look alike but because both types seem to be influenced in the same way by temperature and light. The agent probably is of a chemical nature produced in one case by a chain of events initiated by the plant itself, when it carries certain hereditary genes, and in the other case similar results are produced by interaction of certain fungi with the seed or seedling.

SUMMARY

Corn-seedling virescence, indistinguishable from that caused by certain genetic factors, has been produced by inoculating the seed with either *Aspergillus flavus* or *A. tamarii.* To distinguish these 2 types of virescence with respect to their origin, the terms "genetic virescence" and "induced virescence" have been used.

In both types of virescence chlorophyll deficiency is aggravated by inadequate light and low temperature. Because a fungus, operating beneath the soil, is involved in the production of induced virescence, the soil condition, also, is important. Storing the soil, noncropped for several months before using, and keeping the soil moderately wet while the corn is being grown, were helpful in producing virescence. A number of widely different soil types and of different fertility levels gave somewhat similar results.

Open-pollinated corn varieties of the dent, sweet, pop, flint, and flour types developed induced virescence in a large number of the seedlings under proper conditions. A few highly resistant inbred lines of dent corn were found, but most inbred lines tested were susceptible. In order for the inoculation to be effective in producing virescence it was found necessary to break the seed coat or remove part of it. Shaving off the seed coat from the crowns

of the kernels with a sharp knife before shelling the ear was found to be very effective.

Ten isolations of *Aspergillus flavus* and 2 of *A. tamarii* from various sources were all effective in causing induced virescence, but there were differences in their potency. A number of other *Aspergillus* species and certain other fungi tested failed to give the virescent reaction.

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EXPERIMENTS WITH LIQUID LIME SULPHUR FOR SPRAYING APPLES[1]

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INTRODUCTION

From the time of the first use of liquid lime sulphur as a preventive spray for the control of apple scab (3) both experimenter and orchardist have been confronted with some aspect of the problem of spray injury to foliage and fruit. Following his experiments in 1907, Cordley (3) was originally led to recommend liquid lime sulphur, as a substitute for Bordeaux mixture because it seemed to give better scab control and, at the same time, to eliminate Bordeaux injury in the form of fruit russet. The following year, however, he noted a rather severe injury to the foliage following petal fall and also reports some dropping of fruit. As a result of these observations he

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was led to use a slightly greater dilution of lime sulphur, and to recommend a 1–18 dilution in place of 1–15. The equal efficiency and greater safety of lime sulphur as compared to Bordeaux rapidly gained general recognition, yet the problem of spray injury has remained. It was early recognized by Wallace (7) that many factors could contribute to injury, such as the combination of lime sulphur with insecticides, the health and vigor of the tree, the method of application, as well as certain climatological factors at the time of and subsequent to the application.

Recent trends in spraying have included improved equipment, with much higher pressures, and an increased number of spray applications per season, both of which might be expected to increase injury. There has, however, been a corresponding reduction in strength of lime-sulphur solutions to the point where 1–40 or 1–50 has become almost a standard. Occasionally (1, 2), recommendations of 1–100 for summer applications have been made.

OBJECTIVES AND METHODS

In Pennsylvania, in our efforts to find a spray program that would provide a better balance between scab control and spray injury, we have been led to a further examination of the possibilities inherent in liquid lime sulphur. Some of our plots in 1935 and 1936 (5), (6) had shown that a 1–75 dilution of liquid lime sulphur produced distinctly less injury to foliage and fruit than the standard 1–50 dilution. During the season of 1937 it was our aim to study still further the effects of dilution. Some additional objectives included a study of the effects of 2 different dosages of each dilution selected, to which was added also a study of the effect of excess lime when added to each dilution and to each dosage, as well as a preliminary comparison of daytime *vs.* nighttime applications.

In order to facilitate statistical analysis of the results, the experiment as laid out involved 150 randomized single tree plots. There were 24 treatments and a nonsprayed check, each replicated 6 times. These plots were located in a solid block of Stayman trees, about 20 years old, in the orchard of Henry Baugher in Adams County, Pennsylvania. The various treatments are outlined in table 1.

In further explanation of table 1 it should be stated that all spraying was done with a single nozzle gun from the ground, each tree being covered first from underneath and, finally, from the outside. The random arrangement of the 6 individual trees to be covered with each material necessitated some extra driving. The use of a truck-mounted sprayer with a divided tank, however, made possible the use of 2 different materials at each filling, so that the extra driving did not prove to be a serious drawback. The sprayer was equipped with a 20-gal.-per-minute pump, and the pressure was maintained at 550 lbs. The guns as used to obtain the 2 different dosages were fitted with discs providing a 3/16 in. opening in the "big gun" and a 3/32 in. opening in the "small gun," with whirl plates to match, so that an equally fine spray could be obtained with either gun. In using these

TABLE 1.—*Plot treatment Baugher orchard sprayed in 1937 with liquid lime sulphur of different dilutions, amendments, and dosages*

Plot number	Dilution[a]	Amendment[b]	Dosage[c]
	Day applications throughout season		
1	1– 50 lime sulphur	no lime	big gun
2	1– 50 " "	" "	small gun
3	1– 50 " "	5–100 lime	big gun
4	1– 50 " "	5–100 lime	small gun
5	1– 75 lime sulphur	no lime	big gun
6	1– 75 " "	" "	small gun
7	1– 75 " "	5–100 lime	big gun
8	1– 75 " "	5–100 lime	small gun
9	1–100 lime sulphur	no lime	big gun
10	1–100 " "	" "	small gun
11	1–100 " "	5–100 lime	big gun
12	1–100 " "	5–100 lime	small gun
13	1–200 lime sulphur	no lime	big gun
14	1–200 " "	" "	small gun
15	1–200 " "	5–100 lime	big gun
16	1–200 " "	5–100 lime	small gun
	Day applications in early sprays Night applications beginning with first cover spray		
17	1– 75 lime sulphur	no lime	big gun
18	1– 75 " "	" "	small gun
19	1– 75 " "	5–100 lime	big gun
20	1– 75 " "	5–100 lime	small gun
21	1–100 lime sulphur	no lime	big gun
22	1–100 " "	" "	small gun
23	1–100 " "	5–100 lime	big gun
24	1–100 " "	5–100 lime	small gun
25	Check—no sprays		

[a] Dilutions based on a specific gravity reading of 1.30 for the concentrated lime sulphur.

[b] Lead arsenate at 3 lb. per 100 gal. was used in all applications. Fresh hydrated lime was used in the plots indicated beginning with the petal fall and in all subsequent applications.

[c] Variations in dosage with the first cover spray. Use of the "big gun" resulted in applying 12 gal. per tree per application, while "small gun" applied 6 gal. per tree per application. Previous to the first cover spray the "big gun" was used on all plots.

guns an effort was made to cover each tree in a uniform manner. The dosage as applied by the "big gun" amounted to 12 gal. per tree and by the "small gun" to six gal.

The dates of the various spray applications to the Baugher orchard in 1937 were as follows:

Delayed dormant, April 17; Prepink, not applied; Pink, May 3; Petal Fall, May 19; First Cover, June 1; Second Cover, June 11 and 12; Third Cover, June 23; Fourth Cover, July 16–18.

It was not intended that the prepink application should be omitted, but, at that time, the orchard was so wet that it was impossible to drive through it.

Scab was first noticed on the leaves of the check trees on May 12. In the first week in June some preliminary leaf counts were made showing 3 per cent scab where the 1–50 dilution had been used, 7 per cent at 1–75,

9 per cent at 1–100 and 13 per cent at 1–200, with 49 per cent on nonsprayed checks. At this time there was no noticeable effect either from the use of lime or the variation in dosage, since only the first cover spray had been applied.

As the season progressed other differences began to appear. The trees sprayed with 1–200 lime sulphur without lime showed severe foliage burning. This appeared to be of the type generally ascribed to free arsenic in the spray mixture. Accompanying the injury there was a definite "burn out" of the early scab spots. During the week of July 7–15, just prior to the fourth and last cover spray, detailed counts of scab and leaf injury were made as follows: Five hundred leaves from each of the 150 trees, 75,000 in all were counted for scab, and an additional 500 from each tree were counted for injury. An effort was made to count a true random sample from each tree by selecting terminals and spurs, some from the top, some from the inside, and some from around the outside that could be reached from the ground. The counting was done by 2 people, one working only on scab and the other only on injury. Leaves were recorded as scabby or clean, injured or uninjured; burned out scab spots showing no evidence that the fungus was still alive were counted as clean at that time. No effort was made to record slight and severe injury separately, but simply to get an accurate figure for percentage of foliage injured. The mean percentages of scabby leaves for each treatment, *e.g.*, average of 6 trees or 3,000 leaves is given in table 2. Since scab on the foliage is but an indication of what may be expected on the picked fruit, no effort was made to analyze these figures further. Certain facts, however, seem to be self-evident. The large dosage, as represented by the "big gun" gave better control than the small dosage, in every instance but one. Lime apparently had little effect except in the 1–200 dilution where it was effective in preventing the burn out of scab. A similar effect seems evident in the 1–75 dilution at night, although it is not apparent in the 1–100 dilution at night, a fact for which we have no explanation at present.

TABLE 2.—*Percentage of leaves showing scab in Baugher orchard, July 7–15, 1937*

Dilution	Without lime		With lime 5 lb.–100 gallons	
	Big gun	Small gun	Big gun	Small gun
1– 50 day	4.2[a]	5.2	3.8	4.2
1– 75 day	5.4	5.2	5.6	7.8
1–100 day	6.4	9.8	6.5	9.9
1–200 day	1.3	4.8	10.8	17.5
1– 75 night	2.7	5.1	7.1	7.9
1–100 night	9.9	11.3	7.5	8.7

[a] Each percentage figure represents the average of 3000 leaves, or 500 leaves from each of 6 replicates.

The data on percentage of leaves showing injury, number of scabby apples, and yield are presented in their entirety in table 3. These data

TABLE 3.—*Detailed data on spraying experiments*

Treatment	Percentage of leaves showing injury[a]							Number of scabby apples[b]							Yield: bushels per tree						
	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
1	27.1	20.1	31.8	21.1	29.9	28.9	26.5	7	9	9	1	14	4	7.33	8.1	8.0	12.5	6.0	14.0	7.0	9.2
2	14.7	19.4	23.8	15.6	20.9	19.1	18.9	9	4	7	7	8	19	9.00	15.0	9.0	9.0	11.9	11.0	9.0	10.8
3	27.2	14.1	29.7	29.9	25.7	26.5	25.5	7	0	5	1	6	19	6.30	8.2	6.6	6.0	8.0	7.7	9.0	7.6
4	20.7	17.5	24.7	18.5	28.0	20.3	21.6	11	15	4	17	9	17	12.16	8.9	9.0	10.0	18.0	6.0	14.0	10.9
5	19.7	30.1	15.1	20.0	18.7	30.9	22.4	9	12	11	21	26	13	15.33	8.3	10.0	13.0	12.6	9.0	8.0	10.1
6	6.2	23.7	13.1	16.0	23.7	18.2	16.8	16	8	17	5	5	53	17.33	14.3	10.0	18.0	13.0	10.0	15.5	13.4
7	15.3	16.3	20.4	16.8	18.8	12.7	16.7	14	13	18	9	13	12	13.16	14.0	21.0	18.0	14.0	11.6	16.0	15.8
8	26.1	16.2	15.7	15.1	13.5	10.0	16.1	15	19	78	17	22	20	28.5	14.0	16.0	22.0	11.2	14.0	12.5	14.9
9	14.5	14.6	31.0	16.7	13.7	18.0	18.1	16	20	8	24	31	18	19.50	9.2	17.0	11.0	17.5	19.0	6.0	13.3
10	6.4	12.4	16.4	14.0	19.9	13.5	13.8	29	42	42	27	38	52	38.33	13.7	21.0	11.0	11.4	6.0	15.0	13.0
11	20.8	14.0	12.3	14.1	11.6	15.5	14.7	47	28	49	19	26	14	30.50	9.5	14.0	19.0	16.0	12.0	9.3	13.6
12	14.2	12.1	15.5	21.4	12.3	13.6	14.8	14	30	26	52	57	67	41.00	7.5	14.0	8.0	6.9	15.0	16.0	11.2
13	79.0	87.1	90.4	83.6	82.9	82.6	84.3	51	28	22	26	25	28	30.00	14.0	7.0	7.0	5.0	7.2	8.0	8.0
14	70.3	73.6	67.2	49.6	78.3	75.7	69.1	60	86	77	72	36	39	61.66	10.9	13.0	12.0	10.0	12.0	14.0	11.9
15	17.3	15.4	5.2	12.3	5.9	6.1	10.4	26	43	100	25	64	28	47.66	13.5	13.0	20.6	5.8	18.8	7.0	13.1
16	7.7	9.3	9.9	8.2	12.2	15.0	10.4	84	57	76	102	117	34	78.33	7.5	16.0	14.0	13.0	17.0	4.8	12.0
17	48.6	48.5	51.8	38.9	55.3	42.9	47.7	13	20	4	8	5	2	8.66	12.2	9.0	4.0	11.0	6.9	6.0	8.2
18	35.6	28.0	23.5	37.8	31.2	45.4	33.6	37	14	21	4	22	13	18.51	16.6	9.0	18.0	11.6	14.0	5.0	12.3
19	16.7	18.5	17.7	10.0	16.6	20.0	16.6	17	22	10	18	5	21	15.5	12.0	14.0	15.0	15.8	6.5	9.0	12.0
20	17.0	10.9	19.3	11.8	13.3	6.7	13.2	33	48	24	43	42	48	39.66	11.0	21.0	8.0	15.0	18.0	20.0	15.5
21	12.9	16.2	24.6	20.5	24.2	15.0	18.9	38	50	22	29	33	51	37.16	16.2	19.0	18.0	9.0	8.6	20.0	15.1
22	21.3	18.8	16.5	23.0	21.2	25.4	21.0	65	39	64	28	45	48	48.16	18.0	20.3	20.7	9.5	15.2	16.7	16.7
23	13.2	22.3	13.7	12.6	12.6	14.8	14.9	27	15	15	10	28	44	23.16	12.1	10.0	14.0	14.2	17.0	21.2	14.7
24	6.2	18.0	15.7	11.3	16.6	10.8	13.1	62	56	14	15	34	61	40.33	16.0	12.6	9.0	16.0	13.0	13.4	13.3
25	5.6	5.9	6.0	6.5	10.3	6.4	6.8								1.8	2.0	4.0	6.9	0.0	2.0	2.8

[a] Each percentage is based on a count of approximately 500 leaves. (See text.)
[b] Numbers are based on samples of 300 apples, percentage figures are readily obtained by dividing each number by 3.

TABLE 4.—*Analysis of variance of data presented in table 3*

Analyses and interactions	Foliage injury			Scab on fruit			Yield		
	Degrees of freedom	Sum of squares of deviations	Mean square (variance)	Degrees of freedom	Sum of squares of deviations	Mean square (variance)	Degrees of freedom	Sum of squares of deviations	Mean square (variance)
Preliminary Analysis									
Total	149	50,603.19		143	74,457.23		143	2,719.94	
Treatments	24	47,446.64	1,986.94	23	46,964.89	2,041.95	23	869.53	37.80
Replicates	5	152.19	30.44	5	635.23	127.04	5	60.88	12.17
Remainder	120	3,004.36	25.04	115	26,857.11	233.54	115	1,789.53	15.56
Analysis									
Total	143	48,855.30		143	74,457		143	2,719.94	
Dilutions (D)	5	13,349.28	2,669.85	5	31,555	6,311	5	308.98	61.79
Lime (L)	1	10,309.01	10,309.01	1	1,066	1,066	1	28.09	28.09
Dosage (G)	1	732.64	732.64	1	7,980	7,980	1	62.53	62.53
Interactions									
D×G	5	351.91	70.38	5	2,598	520	5	191.66	38.33
D×L	5	20,345.66	4,069.13	5	3,002	600	5	292.43	58.58
L×G	1	308.80	306.80	1	207	207	1	60.30	60.30
Remainder	125	3,463.00	27.7	125	28,049	224.39	125	1,775.93	14.20

Foliage injury:
Least significant difference between means of Dilutions = 3 per cent.
Least significant difference between means (lime *vs.* no lime) = 0.5 per cent.
Least significant difference between means Dosages ("big gun" *vs.* "small gun") = 0.5 per cent.

Scab on fruit:
Least significant difference between means of dilutions = 8.56 apples or 2.85 per cent.
Least significant difference between means of lime *vs.* no lime = 4.94 apples or 1.65 per cent.
Least significant difference between means of Dosage ("big gun" *vs.* "small gun") = 4.94 apples or 1.65 per cent.

Yield:
Least significant difference between means of dilutions = 2.15 bu.
Least significant difference between means of lime *vs.* no lime = 0.3 bu.
Least significant difference between means of Dosage ("big gun" *vs.* "small gun") = 0.3 bu.

are presented in order to show the amount of variability present in such an experiment, even in a block of trees especially selected for uniformity. An examination of the table will reveal the necessity for statistical analysis in order to determine the significance of comparatively small differences in average percentages.

The 3 sets of data presented in table 3 were subjected to analysis for variance. These analyses are shown in table 4. In each case a preliminary analysis of the data is necessary in order to judge whether significance can be attached to differences between treatments or between the replications or both. From the preliminary analyses it is at once evident that no significance is to be attached to differences between replicates, but that there is a high degree of significance among the treatments. Since the treatments themselves involved 3 variable factors, *i.e.*, dilution, dosage, and lime, some further analysis of the data is desirable in order to determine how much significance may be attached to each separate variable. Omitting the non-sprayed trees as irrelevant to any further analysis and omitting also the replications, already shown to have no significance, and considering the night-sprayed plots simply as additional dilutions, we are able to make another analysis on the basis of 3 criteria of classification, with results as shown further in table 4.

TABLE 5.—*The effects of dilution, dosage and lime on leaf injury and fruit scab, and yield of fruit (comparison of effects of dilutions)*

Dilution	Average leaf injury	Average fruit scabbed	Average yield per tree
	Per cent	*Per cent*	*Bu.*
1– 50 day	23.1	2.9	9.6
1– 75 day	18.0	6.2	14.0
1–100 day	15.3	10.8	12.8
1–200 day	43.5	18.1	11.3
1– 75 night	27.7	6.9	12.0
1–100 night	16.9	12.4	14.8
Check	6.8	86	2.8
Significant difference	3.0	2.85	2.15

In table 5 are shown the mean percentages of leaf injury and fruit scab and mean yield per tree, as influenced by the dilution of the sprays applied. From this table it is evident that the 1–50 dilution produced less fruit, less scab, and more foliage injury than did the 1–75 dilution and that these differences in each case are highly significant. The amount of leaf injury decreases as the dilution increases until a dilution of 1–100 is reached, beyond which point the leaf injury is greatly increased. It seems evident from the work of Hodgkiss and Frear (4) that the effect here is attributable to the liberation of soluble arsenic. The percentage of scabby fruit shows a very consistent relation to the dilution. The differences here shown indicate a very significant increase in scab with each corresponding increase in dilution. It will be recalled that scab on the foliage behaved in a similar fashion

(Table 3). This being true, it seems apparent that if lime sulphur be diluted for the sake of safety, then scab control rapidly becomes a limiting factor. In the case of yield, the results are not so clear cut; yet, it seems certain that both extremes of dilution have resulted in decreasing the yield. So far as night spraying is concerned, there was no significant effect on scab control. At a dilution of 1–75, spraying at night produced more injury than did the same dilution applied in the daytime; yet, with the 1–100 dilution, this effect was not so noticeable. These differences in injury appear also to be correlated with similar differences in yield.

TABLE 6.—*Comparison of effects of dilution vs. dosage*

Dilution	Percentage leaf injury			Percentage fruit scab			Yield, bu. per tree		
	Big gun	Small gun	Diff. in favor big gun	Big gun	Small gun	Diff. in favor big gun	Big gun	Small gun	Diff. in favor big gun
1– 50 day	26.0	20.2	–5.8	2.28	3.52	1.24	8.4	10.9	–2.5
1– 75 day	19.5	16.4	–3.1	4.75	7.63	2.88	12.9	14.2	–1.3
1–100 day	16.4	14.3	–2.1	8.33	13.22	4.89	13.3	12.1	+1.2
1–200 day	47.3	39.7	–7.6	12.94	23.33	10.39	10.6	12.0	–1.4
1– 75 night	32.1	23.3	–8.8	4.02	9.69	5.67	10.1	13.9	–3.8
1–100 night	16.9	17.0	+0.1	10.05	14.75	4.73	14.4	15.0	–0.6
Average	26.4	21.8	*–4.6*	7.06	12.20	*4.96*	11.6	13.0	*–0.6*
	[a] Significant difference = 0.5			Significant difference = 1.65			Significant difference = 0.3		

[a] Figures for significant differences apply only to the differences between the averages. Further analysis of covariance would be necessary to determine the significance of the individual differences at the several dilutions.

In table 6 it can be readily seen that the larger dosage in the cover sprays, as represented by the "big gun" was productive of greater foliage injury and better scab control. The correlation between dosage and dilution is very marked in both cases. It also is apparent that both these factors find

TABLE 7.—*Comparison of effects of dilution vs. lime*

Dilution	Percentage leaf injury			Percentage fruit scab			Yield, bu. per tree		
	Lime	No lime	Diff. in favor lime	Lime	No lime	Diff. in favor lime	Lime	No lime	Diff. in favor lime
1– 50 day	23.5	22.7	–0.8	3.1	2.7	–0.4	9.3	10.0	–0.7
1– 75 day	16.4	19.6	3.2	6.9	5.4	–1.5	15.3	11.8	3.5
1–100 day	14.7	15.9	1.2	11.9	9.6	–2.3	12.3	13.1	–0.8
1–200 day	10.4	76.7	66.3	21.0	15.3	–5.7	12.6	10.0	2.6
1– 75 night	14.7	40.6	25.9	9.6	4.5	–5.1	13.8	10.3	3.5
1–100 night	14.0	19.9	5.9	10.6	14.1	3.5	14.1	15.9	–1.8
Average	15.6	32.2	*16.6*	10.5	8.6	*–1.9*	12.9	11.8	*1.1*
	[a] Significant difference = 0.5 per cent			Significant difference = 1.65 per cent			Significant difference = 0.3 bu.		

[a] Figures for significant differences apply only to the differences between the averages. Further analysis of covariance would be necessary to determine the significance of the individual differences at the several dilutions.

additional expression in terms of yield. Here, it would seem, spraying was more detrimental than beneficial unless we keep in mind the performance of nonsprayed treees.

Turning next to the effects of lime when added to the various dilutions as summarized in table 7, it is at once evident that lime has a very beneficial effect, so far as reducing injury is concerned. This effect is most pronounced in the extreme dilutions. That it also permits a greater build up of scab is readily recognized, and that, in general, it has produced a better yield seems not to be denied.

DISCUSSION

The field experiment in orchard spraying here outlined is, first of all, illustrative of methods that, it is believed, have been all too little used by plant pathologists interested in the control of fruit diseases. The senior writer first used this type of experimental procedure in his own work in 1935 (5), and has long felt that in experimental field work involving so many controversial points as does orchard spraying, the investigator can ill afford to leave any stone unturned that might contribute to his efforts to secure accurate, unbiased, quantitative information. Following the season of 1937, we are more than ever convinced that a randomized-plot layout, such as we have here employed, can be used to advantage and that the extra driving and labor involved in the physical application of materials are worth the effort.

No problem so complicated as orchard spraying, involving as it does an attempt to balance disease control against possible injury, can be solved in one year's field work. Indeed, it is possible in view of the ever increasing list of new materials, new equipment, ever-changing seasonal conditions, etc., that it may never be solved to the satisfaction of either grower or investigator. Yet, at the same time, progress can be made and should be recorded in such manner as to build an ever-firmer foundation for field practices.

It should, perhaps, be pointed out that the data on foliage injury do not give a complete picture of the injury. While the percentage of injured foliage has been recorded, the relative areas of injured leaf surface are not apparent from the figures. Thus the injury recorded for the 1–200 dilution without lime, was actually more severe than even the high percentage of injured leaves would indicate, on account of the large area of leaf surface involved. Conversely, the 1–75 dilution actually appeared in the field to much better advantage than the percentage figures would indicate because, for most of the leaves recorded as injured, the amount of leaf surface involved was relatively very small. It would have been of interest and value to record such injury in terms of the relative sizes of the injured areas, but time and labor at our disposal did not permit such detailed records in this instance.

While we are not here concerned primarily with recommendations, it does seem evident that a lime-sulphur dilution of approximately 1–75, if used throughout the season, offers a better chance of adequate scab control, together with a minimum injury, than either a stronger or a weaker solution. While the stronger solution gives better scab protection, and the weaker solution with additional lime may be slightly safer, the 1–75 dilution provides a better balance when both control and injury are considered.

SUMMARY

Studies in orchard spraying are described that make use of replicated, randomized single tree plots, and permit analysis of variance of the results.

These studies deal with dilutions of liquid lime sulphur, together with such factors as dosage and the addition of hydrated lime, and the effects of these upon foliage injury, fruit scab and tree yield.

Scab has been shown to increase progressively with the dilution. Foliage injury, on the other hand, shows a tendency to decrease with increased dilution up to 1–100, and to increase sharply beyond that point.

Of two dosages used, the larger resulted in greater injury and better scab control, but decreased yield.

The use of lime with the various dilutions and dosages is shown to reduce injury and increase yield, but to permit a slightly greater development of scab.

When foliage injury, scab and yield are all considered a dilution of 1–75 provides the best balance between injury and scab control.

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SUSCEPTIBILITY OF NEEDLES OF DIFFERENT AGES ON PINUS MONTICOLA SEEDLINGS TO CRONARTIUM RIBICOLA INFECTION

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REVIEW OF PREVIOUS STUDIES OF PINUS STROBUS AND P. MONTICOLA

Since Clinton and McCormick first demonstrated in 1917 that white pine blister rust (*Cronartium ribicola* Fischer) enters its aecial host through the needles (3), considerable interest has been evidenced as to possible differences in susceptibility between needles of various ages and the influence of such differences on the cankering of pines. From their work on eastern white pine, *Pinus strobus* L., Clinton and McCormick concluded, although positive evidence was lacking, that the sporidial germ tubes gain entrance into the needles through the stomata (3, p. 449). Accepting this conclusion, Spaulding (14) attempted a possible explanation of the differences in susceptibility between species of white pine on the basis of needle persistence and distribution of stomata. In a recent publication by Hirt (5), it is shown that the sporidial germ tubes are capable of penetrating directly through the epidermis and it is suggested that this, as is true of most other Uredinales (1, p. 229; 2, p. 257), may be the usual mode of entrance.

The results of studies on eastern white pine have not been entirely consistent, but, in general, have shown needles of the current and second seasons to be about equal in susceptibility. In some cases, however, there are indications that needles of the current season may be slightly more susceptible. Differences in methods employed by investigators may partly explain the lack of uniformity in results.

Various methods of inoculation employed by Clinton and McCormick in 1917 and 1918 consistently resulted in the highest percentage of infection on 1-year-old pines (3, p. 445).

In 1921 York and Snell (15) were successful in inoculating potted seedlings of *Pinus strobus* of the seasons 1918 and 1921. A fascicle of needles from a 1918 seedling and one entire 1921 seedling were sent to Dr. R. H. Colley for critical examination. He reported to them, in part: "If anything, there are proportionately more individual infections in the needles of the 1921 seedling than in full grown leaves."

Inoculations made by Snell and Gravatt in 1922 (12, p. 589) were as successful on 2-year-old needles of *Pinus strobus* as on needles but 1 year old.

In a later paper (16), the above 1921 and 1922 inoculations are discussed further. In comparable 1921 experiments 77 per cent of the year-old seedlings became infected, whereas only 58 per cent of the older ones (mostly over 3 years old) became infected (p. 502). In the 1922 inoculation of potted pines, seedlings of all ages except year-old ones became infected (p.

505). Quoting directly from York *et al.* (16, p. 507): "Clinton (2) and the writers (7, 17) stated that both the first and second year needles of *Pinus strobus* may become infected, but they gave no data on the relative susceptibility of needles of different ages. In an attempt to get some definite information on this point in 1922, 60 inoculations were made on needles of known ages on older trees. Two per cent of these inoculations were made on 1920 needles, 18 per cent on 1921 needles, and 80 per cent on 1922 needles. None of the inoculations on 1920 needles, 18 per cent of those on the 1921 needles, and 19 per cent of those on the 1922 needles developed leaf spots. None of the inoculations on 1920 needles, 9 per cent of those on 1921 needles, and 8 per cent of those on the 1922 needles resulted in twig cankers. These results agree with the statement previously made that 1921 and 1922 needles seemed of approximately equal susceptibility (7)."

From observations made in 1920 Richards (9) concluded that the relative susceptibility of eastern-white-pine needles to blister rust infection increases with the age of the needles. It must be realized, however, that the exact date of infection was impossible to determine.

From an analysis of 15,000 cankers in the Adirondacks, Snell (10, p. 479) reasoned that in all probability many cankers originate on 2- and 3-year-old needles as well as on those of the current season.

In 1927 Hirt (4) exposed 3-year-old transplants of *Pinus strobus* to sporidial infection on *Ribes nigrum* L. for a 24-hour period before removal to a permanent planting area. Upon the trees that became infected as a result of this exposure over 900 cankers developed, approximately two-thirds of which appeared on 1926 wood and the remainder on 1927 wood.

Snell (11) reports, for the first time, the results of two series of inoculation experiments on *Pinus strobus* that yielded contradictory results, one showing the current-season needles to be higher and the other showing them to be lower in susceptibility than the older needles. In this same paper he summarizes previously published evidence from which he concludes, in effect, that the current-season needles of *P. strobus* are to a greater or less degree more susceptible to *Cronartium ribicola* than are the second-season needles, although he does not consider the question incontrovertibly settled.

The results of studies conducted in a manner similar to that employed by the authors have recently been published by Hirt (5). Working with *Pinus strobus* he found that the current-season needles are only about one-fourth as susceptible as year-old needles (p. 189).

Needle susceptibility data for western white pine, *Pinus monticola* Dougl., have not been previously determined by precise inoculation experiments. From an analysis of approximately 6,000 cankers on both large and small trees, however, Lachmund (6, 7) offers very convincing evidence that the current-season needles of this species are relatively resistant to blister-rust infection. In one of these papers Lachmund (6) stresses the importance of this resistance in studies wherein it is desirable to determine the year of origin of established waves of pine infection.

In 1933, inoculation experiments were conducted near Bovill, Idaho, with small potted western white pines. This paper describes these experiments, which were conducted as a check on the validity of Lachmund's (7, p. 920) previous conclusion that the current-season needles of *Pinus monticola* are relatively resistant to infection by *Cronartium ribicola.*

TEST PLANTS[1]

Forty-six thrifty *Pinus monticola* seedlings from 5 to 7 years of age and from 7 to 15 inches in height were selected as test material from a presumably rust-free area near Bovill, Idaho, on September 7, 1933. Careful examination of these trees had failed to disclose any cankers or any needle spots that might have been blister-rust infections. Fall needle cast was complete on all trees and the remaining needles gave evidence of remaining attached for the duration of the study. The trees were carefully dug and then transported in wet moss to Deep Creek, near the town of Elk River, Idaho, where they were immediately potted in native forest soil.

Thirty-four of these potted pines were exposed to infection by *Cronartium ribicola* in the manner described in the following section. The 12 remaining trees served as check plants and were transported back to the Bovill area immediately after potting and before being exposed to infection.

EXPOSURE OF PINES TO INFECTION

On September 7, 1933, the 34 potted pines were placed in trenches dug in the moist sand bordering Deep Creek. Dense concentrations of native *Ribes petiolare* Dougl., a common stream-type species of high susceptibility and telium-producing capacity (8), formed an almost closed leafy canopy over the entrenched pines. The lower leaves on the bushes were bearing very heavy blister-rust infection in the telial stage. The uppermost leaves bore well-advanced uredial infection that showed promise of soon developing telia, thus insuring a continual supply of viable sporidia to the pines.

Rainfall occurred intermittently over the white-pine belt of northern Idaho between September 9 and 24, inclusive. The experiment at Deep Creek was being conducted in the bottom of a damp, well-shaded canyon, making conditions for sporidial production and pine infection favorable throughout the entire 16-day period. Climatic conditions after September 24 were not so favorable for pine infection. Severe frosts occurred in the last week of September and when visited on October 4 the *Ribes* plants surrounding the potted pines were completely defoliated.

On October 11 the 34 exposed pines and the 12 check plants left near Bovill were removed to a greenhouse[2] at Moscow, Idaho. The 34 pines, then, had been in close proximity to infected *Ribes* for a total of 35 days (Sept. 7 to Oct. 11). Spaulding (13) has shown, for certain other *Ribes* species, that when plants are defoliated by autumnal frost the telia may remain viable for

[1] The pines used in these experiments were made available through the courtesy of the Office of Blister Rust Control, Spokane, Washington.

[2] Greenhouse and laboratory space were provided through the courtesy of the School of Forestry, University of Idaho.

several weeks on the fallen leaves. In view of his findings it seems logical to assume that the pines in this study were exposed to infection throughout the entire 35-day period, although there were no leaves on the *Ribes* plants after October 4. During only 16 of these days (Sept. 9 to 24), however, were climatic conditions generally favorable for pine infection.

CARE AND EXAMINATION OF PINES

Once in the greenhouse, the group of 34 exposed pines and 6 of the check plants were kept at a temperature nearly the same as that outside. The 6 remaining check plants were left in a cold frame out-of-doors. Careful observations were made daily in order to detect the first visible evidences of needle infection. The first minute needle spots became visible on November 9, 1933, and from then until November 16 hundreds of spots appeared daily on all ages of needles. One month was allowed, thereafter, for further incubation and an analysis of the infection was then made. Thus 66 days had elapsed since the pines were last exposed to infection, allowing ample time for at least the majority of the infection spots to become visible on needles of all ages. Clinton and McCormick (3, p. 442) found that needle spots become visible to the naked eye a month or two after inoculation.

When the first rust spots began to appear on the needles of the exposed seedlings identification was made positive by microscopic examination. Characteristic blister-rust mycelium (3, pp. 450–451) was found in abundance within the needle tissues, particularly in the mesophyll area. Later, as the needle spots acquired their characteristic appearance and translucent lemon-yellow color (3, plate 40; 16, plate 1), identification was made macroscopically and occasionally verified microscopically. An examination of the 12 check plants failed to reveal any needle spots that could be identified as white-pine blister-rust infections.

In view of the variation encountered in western-white-pine needles it was considered most accurate to present the final susceptibility data on a basis of needle area. Needle area was computed from the formula: Area = N × L (C + 10r), in which N = number of fascicles on a given internode, L = length of needles, C = circumference of fascicle at the base, and r = radius of fascicle. The relation of C and r to length of needle fascicle was determined from measurements of 355 fascicles collected on seedlings from the locality where the test plants had been secured. In computing area of foliage on the test plants it was only necessary to measure fascicle length and then enter that value and the corresponding C and r values in the formula. No allowance was made for the tapering of the needles.

RESULTS

Table 1 shows the relative susceptibility of needles of different ages to white-pine blister rust in terms of number of infection spots per 100 square centimeters of foliage surface exposed. From this table it is strikingly evident that the current-season needles are decidedly lower in susceptibility than are the older needles. This relationship holds true irrespective of length

TABLE 1.—*Needle spots developed on potted 5- to 7-year-old Pinus monticola seedlings from exposure to infection by Cronartium ribicola on native Ribes petiolare from September 7 to October 11, 1933*

Length of time needles retained	Trees	Year's wood bearing needles	Needles	Surface area of needles		Needle spots		Needle spots per 100 square centimeters of surface area
				Actual	Per cent of total	Actual	Per cent of total	
(*Years*)	(*No.*)		(*No.*)	(*Sq. cm.*)		(*No.*)		(*No.*)
		1933	895	941	51.4	8	4.0	.9
4	4	1932	685	401	21.9	67	33.7	16.7
		1931	410	433	23.6	117	58.8	27.1
		1930	79	57	3.1	7	3.5	12.4
		1933	7,390	7,835	55.0	90	7.2	1.2
3	20	1932	4,445	5,162	36.3	956	77.0	18.5
		1931	938	1,236	8.7	196	15.8	15.9
		1933	1,940	2,348	59.0	48	12.3	2.0
2	10	1932	1,405	1,649	41.0	341	87.7	20.7
	34	1933	10,225	11,124	55.4	146	8.0	1.3
Totals and	34	1932	6,535	7,212	36.0	1,364	74.5	18.9
averages	24	1931	1,348	1,668	8.3	313	17.1	18.8
	4	1930	79	57	.3	7	.4	12.4

of time the needles are retained. Combining the results for all trees tested, needles of the second and third seasons are found to be essentially equal in susceptibility. Fourth-season needles, although poorly represented, are somewhat lower in susceptibility than second- and third-season needles but decidedly higher than current-season needles.

Although the exact date of infection resulting in a given needle spot could not be determined the minimum incubation period was not less than 29 days (last possible date of infection Oct. 11; first needle spot appeared Nov. 9). The most probable incubation period, however, was between 46 and 68 days. (Most favorable infection period Sept. 9 to 24; majority of needle spots appeared Nov. 9 to 16).

For the complete picture from needle infection to canker development it would be necessary to know the number of fascicles bearing infected needles on internodes of various ages and also just how many of these infected fascicles remain attached sufficiently long to permit the fungus mycelium to grow down the needle and become established in the bark of the twig. It must be kept in mind that the study herein reported was concerned only with the relative susceptibility of needles of different ages and that no consideration was given to subsequent canker development. The salient feature of this study was the determination that needles of the current season are relatively low in susceptibility to infection by white-pine blister rust and that needles of the second and third seasons are relatively high in susceptibility. Lachmund's scale of canker distribution (6, pp. 689–690, 692) shows that relatively few cankers form on internodes of the current season, whereas the majority form on internodes of the second and third seasons. The relative susceptibility of needles of different ages corresponds, in general, to the relative abundance of cankers formed on internodes of different ages. From this comparison it seems logical to conclude that needle susceptibility is a major factor governing the distribution of cankers on internodes of various ages.

SUMMARY

Thirty-four potted native *Pinus monticola* seedlings ranging from 5 to 7 years of age and from 7 to 15 inches in height were exposed to *Cronartium ribicola* infection on native *Ribes petiolare* bushes from September 7 to October 11, 1933, at Deep Creek, Idaho. After a minimum incubation period of 66 days the resultant needle spots were tallied and summarized on the basis of age of needles exposed. From this tally it was apparent that needles of the current season are relatively low in susceptibility, needles of the second and third seasons relatively high and approximately equal in susceptibility, and needles of the fourth season intermediate but still quite high in susceptibility.

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STUDIES ON POLYPORUS ABIETINUS

I. THE ENZYME-PRODUCING ABILITY OF THE FUNGUS[1]

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INTRODUCTION

The activity of wood-destroying fungi is conditioned by their ability to form a variety of enzymes. This fact has been demonstrated by several investigators, among whom are Schmitz and Zeller (16), working with *Polyporus lucidus* (Leys.) Fries (*Ganoderma lucidum* (Leys.) Karst.), *Armillaria mellea* (Vahl.) Fr., and *Daedalea confragosa* (Bolt.) Fr., and Zeller (19) working with *Lenzites saepiaria* (Wulf.) Fr. The object of the present study, therefore, was to determine the kinds of enzymes produced by the coniferous sapwood-destroying fungus *Polyporus abietinus* (Dicks.) Fr.; to compare the enzyme-producing ability of this fungus with that of

[1] A portion of a thesis submitted to the Graduate Faculty of Duke University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] The author herewith gratefully acknowledges his indebtedness to Dr. Frederick A. Wolf, under whose guidance this work was conducted.

other xylophilous fungi; and to compare 2 methods of demonstrating the production of enzymes.

METHODS

The methods for determination of enzymes used in this study are: (a) the *in vitro* method, in which an extraction of the fungal hyphae is added to solutions of various substrates. After incubation, tests are made for the presence of appropriate end products; and (b) the *in vivo* method, developed by Crabill and Reed (5), in which the fungus is cultured on a medium containing a single organic nutritive material. Either the growth of the organism or some change shown by the indicator is regarded as proof that the fungus produces the enzyme necessary for utilization of the organic substance.

In the beginning of the study a vigorous polysporic culture of the fungus was isolated by inverting a sporophore over malt agar. Transfers from this original culture were used throughout the study.

For the *in vitro* tests the fungus was grown for 3 months, in flasks, on autoclaved loblolly pine sawdust. The contents of the flasks were then triturated with sterile sand, and allowed to extract overnight in cold water. After filtration, the enzymes were precipitated from this solution with 2 volumes of 95 per cent alcohol. This preparation was filtered through a Buchner funnel, and the precipitate on 3 sq. cm. of filter paper constituted the enzymic extract from 20 g. of material. Strips, 3 sq. cm. in area, of this filter paper with the adhering enzymic extract were placed in test tubes with 2.5 per cent solutions of the substances listed in table 1, and incubated at 30° C. for the indicated periods. Toluol was used as an antiseptic, and, unless otherwise indicated, the usual methods for determination of end products were used.

In a few cases the methods of testing for enzymic activity were different from standard methods and deserve further mention. In the tests for lipase the substrate was alkalized with sodium hydroxide, and brom-thymol-blue was added as an indicator. Under these conditions the activity of lipase would result in the formation of acids, changing the indicator from blue to yellow. The same procedure was followed in testing for peptic activity. In testing for tryptic activity the blood fibrin was stained with Congo red, and fixed with hot water. Release of the dye is, in this case, an indication of the action of trypsin. The tryptophane test was applied in testing for the activity of erepsin. In tests for urease and asparaginase, rosolic acid was added to the substrates, and, if reddening of the solutions occurred, it indicated the production of ammonium by the activity of these enzymes. In the tests for maltase, lactase, and tanninase, the Barfoed method of testing for monosaccharides in the presence of reducing disaccharides was used in preference to the complex Schaffer method. In the test for activity of tanninase the solution was precipitated with albumin, and neutralized with excess sodium hydroxide before testing for monosaccharides.

Two types of controls were set up in this series of tests: (a) autoclaved strips of the filter paper with the adhering precipitated enzymes were placed in contact with the substrates, and (b) strips of filter paper on which no precipitated enzymes were suspended in similar substrates. The controls were incubated along with the tests.

The filter paper itself constituted the substrate in the tests for cellulase and cellobiase. In this case the filter-paper strips, with the adhering precipitated enzymes, were suspended in distilled water. A series of tubes was prepared sufficient to make tests with Fehling's solution at intervals of 12 hours. As a result it was determined that no appreciable hydrolysis of the cellulose had occurred prior to 12 days. The extended period required for the production of reducing sugars by the hydrolysis of cellulose makes it posssible to avoid misinterpreting the results in all other tests in which cases reducing sugars appeared rather promptly.

In the application of the *in vivo* tests, 2.5 per cent of each of the materials listed in table 1 was added to a mineral nutrient stock medium. This stock medium was composed of the following:

$MgSO_4$, 0.5 grams; KCl, 0.5 grams; K_2HPO_4, 1.0 grams; $FeSO_4$, trace; Agar, 20 grams; Water, 1000 cc.

Plates were poured with these stock media plus the various materials and were inoculated with *Polyporus abietinus*.

In tests in which negative results were obtained with this stock medium, the tests were repeated with the same medium enriched by the addition of 2.9 g. of $NaNO_3$. Tests in which both media were used are indicated with an asterisk.

Only the *in vitro* method was used in testing for oxidizing enzymes.

RESULTS

A summary of the results of these tests to determine the enzyme-producing ability of *Polyporus abietinus* is presented in table 1.

DISCUSSION

In this study positive evidence was obtained for the formation of 19 kinds of enzymes by *Polyporus abietinus*. Negative results were obtained in the tests for the hydrolytic enzyme lactase, and for the oxidizing enzyme tyrosinase. With the exception of Zeller's study (19), this list of enzymes is more extensive than lists previously reported for wood-destroying fungi. Moreover, the kinds of enzymes produced by *P. abietinus* are essentially the same as by *Lenzites saepiaria* listed by Zeller. Since both *P. abietinus* and *L. saepiaria* are potent destroyers of coniferous sapwood, it might be expected that they would be able to form similar enzymes.

An appreciation of the enzyme-forming ability of *Polyporus abietinus* can best be gained by comparison with the published results of similar studies involving other wood-inhabiting species. These comparisons can be presented most succinctly in tabular form in table 2.

TABLE 1.—*Enzymes produced by Polyporus abietinus, grown on a mineral nutrient stock medium of $MgSO_4$, KCl, K_2HPO_4, $FeSO_4$, agar, and H_2O*

Enzyme tested for	Substrate	Incubation *in vitro*	Period, days *in vivo*	Results *In vitro*	Results *In vivo*
Sucrase	Sucrose	3	7	+	+
	Raffinose	3	9	+	–
Maltase	Maltose	14		+	
	Amygdalin	7	26	+	–
Emulsin	Raffinose	7	26	+	–
	Salicin	7		+	
Cellulase (and cellobiase)	Filter paper cellulose[a]	14	9	+	+
Lactase	Lactose[a]	14	7	–	–
Amylase	Corn starch	3	7	+	+
Inulase	Inulin[a]	3	7	+	+
Pectinase	Pectin		9	+	–
Lipase	Methyl acetate	5	14	+	+
Pepsin	Blood fibrin	5	26	+	–
Trypsin	Blood fibrin	14	26	+	–
Erepsin	Peptone	14	26	+	–
Urease	Urea	4			+
Asparaginase	Asparagin[a]	4	7	+	+
Tanninase	Tannic acid	5	7	+[b]	+
Ligninase	Isolated lignin[a]		26		+
Oxygenases	10% alcoholic gum guaiac	½		+	
Laccase	Tannic acid	½		+	
Tyrosinase	Tyrosine	10		–[c]	
Peroxidase	Gum guaiac and H_2O_2	1 hour		+	
Catalase	H_2O_2	½ hour		+	

[a] Test repeated on mineral nutrient stock medium enriched by addition of 2.9 g. of $NaNO_3$.
[b] + indicates positive evidence.
[c] – indicates negative evidence.

Tests were made by Zeller (19) with *Lenzites saepiaria,* and in the present study with *Polyporus abietinus* for a larger number of enzymes than is the case with any of the other fungi. Certain of them, if appropriate tests were made, might be found to form other kinds of enzymes. The assembled data do indicate, nevertheless, that there is considerable variation in enzyme-producing ability among xylophilous fungi, particularly in reference to sucroclastic and proteolytic enzymes. Table 2 further reveals that *P. abietinus, L. saepiaria,* and *Daedalea confragosa* are quite diversified in their enzyme-producing ability. They secrete several enzymes not formed by species of heartwood-destroying fungi, such as *Polyporus squamosus, P. sulfureus,* and *Echinodontium tinctorium,* nor by the highly specialized cambium-attacking fungus *Armillaria mellea.*

The evidence obtained herein for the production of both cellulase and ligninase by *Polyporus abietinus* corroborates the results of Campbell (4) who found that coniferous sapwood decayed by this fungus is lower in both cellulose and lignin than is undecayed wood.

With the exception of inulase, all of the hydrolytic enzymes demonstrated for *Polyporus abietinus* are necessary for the complete utilization of some

TABLE 2.—*Kinds of enzymes produced by various wood inhabiting species of Basidiomycetes in comparison with those produced by Polyporus abietinus*

Investigator	Organism	Enzymes																				
		Emulsin	Cellulase	Ligninase	Amylase	Sucrase	Maltase	Lactase	Inulase	Pectinase	Tannase	Pepsin	Trypsin	Erepsin	Urease	Asparaginase	Lipase	Oxygenase	Peroxidase	Tyrosinase	Laccase	Catalase
The writer	Polyporus abietinus (Dicks.) Fr.	+[a]	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Schmitz (14)	Polyporus volvatus Pk.	+	+		+	+	+	+	+		+	−		−	−		+					+
Buller (3)	Polyporus squamosus Huds.	+	−		+	−	−					+					+			+	+	
Bayliss (1)	Polyporus versicolor (L.) Fr.	−[b]	+	+	+	+					+	+		+			−			+		
MacDonald (9)	Polyporus betulinus (Bull.) Fr.	+			+	−	−				+	+		−			+			+	+	
Nutman (11)	Polyporus hispidus (Bull.) Fr.	+	+	+		+												+				+
Bourquelot and Herissey (2)	Polyporus sulfureus Bull.	+	+		−	−	+		−		+											
Schmitz and Zeller (16)	Polyporus lucidus (Leys.) Fr.	+	+			+	+	+	+		+		+	+	+		+	+				
Schmitz (14)	Fomes igniarius (L.) Gill.	+	+		+	+	+	+	+		−	+			+		+					+
Schmitz (15)	Fomes pinicola Fr.		+		+	+	+	+	+		+				−		+					+
Montgomery (10)	Fomes fraxineus (Bull.) Cooke	+	−		+	+				+							+	+	+			+
Schmitz and Zeller (16)	Armillaria mellea (Vahl) Fr.				+	+	−	−	+								−	+	+			+
Schmitz and Zeller (16)	Daedalea confragosa (Bolt.) Fr.	+			+	+	+	+	+		+		+	+	+		+	+				
Schmitz (13)	Echinodontium tinctorium E. & E.		+		+	+	+	+	+			−		−	+		+					+
Zeller (19)	Lenzites saepiaria (Wulf.) Fr.	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+			+
Findlay (6)	Paxillus panuoides Fr.	+	+		+																	
Putterill (12)	Schizophyllum commune Fr.	+		−	+	+											+					+
Venkataravan (17)	Ganoderma lucidum (Leys.) Karst.	−				+	−					+									+	−

[a] + indicates positive evidence.
[b] − indicates negative evidence.

material found in coniferous sapwoods. In spite of the fact that certain of these materials (glucosides, sucrose, and proteins) are found in minute quantities, it is shown that the fungus is able to utilize them. Consequently, it is evident that materials stored in the parenchyma cells of the sapwood are of importance in the nutrition of this fungus. Wilson (18) has shown that the amount of food stored in parenchyma cells of wood influences the liability of attack of wood by insects and sap-staining fungi. In view of the present evidence, it is equally logical to assume that the amount of stored food likewise influences the rate of growth of sapwood-destroying fungi.

Of the 21 enzymes tested for in this study, 7 were demonstrated by the *in vitro* method, which could not be shown by the *in vivo*. Recently, however, the determination of plant enzymes *in vitro* has been subjected to considerable criticism. The more valid objections to the *in vitro* method, as discussed by Kertesz (7) are: (a) the possibility that the treatment during extraction diminishes or inhibits the activity of enzymes; (b) the possibility that, when extracted from the cells, the proteolytic enzymes may digest some of the other enzymes; (c) the possibility that all enzymes may not be soluble in the solvent used, and, hence, may not be extractable; (d) the difficulty of establishing that there has been no bacterial activity during the extraction or in the substrate.

It is obvious that none of these objections may be leveled at the *in vivo* method are herein employed. It would seem that an easily-cultured saprophytic fungus affords an excellent opportunity for demonstrating enzymatic activity in vigorously growing material. In the majority of tests in which the results from the 2 methods are contradictory, it is evident that the single organic material used, even though hydrolyzed, would not support growth and activity of the fungus. The employment of the *in vivo* method, with appropriate modifications, as a substitute for the *in vitro* method for demonstrating enzymatic activity is, nevertheless, highly desirable in future investigations with microorganisms.

SUMMARY

The coniferous-sapwood-destroying fungus *Polyporus abietinus* is herein shown to form 15 of the more common hydrolytic enzymes, namely, emulsin, cellulase, ligninase, amylase, sucrase, maltase, inulase, pectinase, tanninase, pepsin, trypsin, erepsin, urease, asparaginase, and lipase; and is shown to form 4 of the more common oxidizing enzymes, namely, oxygenase, peroxidase, laccase, and catalase.

Polyporus abietinus produces more kinds of enzymes than have been previously reported for other wood-destroying fungi, with the exception of *Lenzites saepiaria*.

The variety of hydrolytic enzymes produced by *Polyporus abietinus* indicates that materials stored in the wood parenchyma are of considerable importance in the nutrition of the organism.

A comparison of results obtained by the *in vitro* and *in vivo* methods indi-

cates that the former more accurately demonstrates total number of enzymes that a given microorganism is capable of producing.

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HOST-PARASITE RELATIONS OF SCLEROSPORA GRAMINICOLA ON SPECIES OF SETARIA[1]

E. S. McDonough[2]

(Accepted for publication July 15, 1938)

Infection of *Setaria italica* (L.) Beauv., *Panicum miliaceum* L., *Euchlaena mexicana* Schrad., and *Zea mays* L. with germinating oospores of *Sclerospora graminicola* (Sacc.) Schroet. was first reported by Melhus and Van Haltern (3) in 1925. Later, Melhus, Van Haltern, and Bliss (4) reported infecting species in 5 genera of the Gramineae with *S. graminicola,* and that infection of the seedlings took place between germination and emergence. Since this early work, numerous investigators have demonstrated the infectious nature of *Sclerospora* oospores on a wide range of host plants.

Hiura (1) placed germinating oospores directly on the young plant parts, and reported that infection took place through either the coleoptile, the root, or the mesocotyl. Oospores were reported capable of causing infection within 4 days after they had been sown. The hyphae were reported to have penetrated the epidermis of the host. Such a penetration, however, was not illustrated nor was it described in detail.

The present study on the host relationship of *Sclerospora graminicola* on *Setaria viridis* and *S. italica* was initiated in 1935. The entrance of the infection hypha emanating from the oospore into the host and some observations on the subsequent development of the parasite were reported in 1937 (2). In the investigation here reported an attempt was made to study the growth of the mycelium initiated by the oospore until a systemic infection was established, the tissues of the host involved, and the general relations of the fungus to its host.

METHODS AND MATERIALS

Shredded leaves of *Setaria viridis* plants infected with *Sclerospora* were collected near Ames, Iowa, in October of 1935, near Waukesha, Wisconsin, in March, 1936, and near Milwaukee, Wisconsin, in October, 1936. Care was taken to select leaves containing oospores that had turned a reddish brown. In all instances the plants had not lodged and the leaves were thoroughly dry. Preliminary examinations of oospore material indicated that the oospores from different localities and even from parts of the same plant varied widely in the percentage of germination. For this reason only material showing the most uniformly high percentage of germination was used in infection experiments.

[1] The results reported here were obtained at Iowa State College and at Marquette University. The original report was included in a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his appreciation to Dr. J. E. Sass under whose direction the investigation was carried out and to Dr. I. E. Melhus who suggested the problem and who gave invaluable advice during the course of the work.

The following method was used to expose seedlings to infection: The seed was placed on moist cotton in a Petri dish, the cover of which also had been lined with moist cotton. Such seed was then covered with shredded leaf material containing the oospores and allowed to stand at room temperature. One hundred hours after the seed had been sown, the Petri dishes were transferred to bell jars near a window, and the lids removed, care being taken to keep the cotton moist. Small beakers of water were placed under the bell jars with the Petri dishes. Conidia usually were observed on susceptible plants 5 days after the seed had been covered with the oospores. Preliminary observations of infection were made with material stained and fixed in aceto-carmine.

Artificially infected material that was to be sectioned in paraffin was killed in standard chromo-acetic and in a solution containing 75 ml. of 1 per cent acetic acid, 20 ml. of 1 per cent chromic acid and 5 ml. of 37 per cent formaldehyde. By careful dissection under a binocular microscope it was possible to obtain very young, whole seedlings for preservation. The fertile lemma was removed from the seed with a dissecting needle. As soon as the lemma had been removed, the seedling was carefully cut from the fruit and dropped into the fixing fluid. Very little shrinkage of material was observed, probably because of the rapidity with which the dissection was made and also because of the more or less noncutinized condition of the seedling.

EXPERIMENTAL RESULTS

In March, 1936, seeds of *Setaria italica* (Siberian variety) were exposed to infection. As a rule the coleorhiza was the first part of the seedling to emerge, although under exceptional conditions the coleoptile was first. As soon as the coleorhiza had emerged, the young seedlings from one lot were dissected out of the fruit and preserved. Twenty-four hours later the roots of the remaining plants averaged 4 mm. in length, and the seedlings from another dish were dissected out and fixed. Two more dissections were made at intervals of 24 hours. Fourteen days after the seeds had been sown the seedlings were cut off just above the place where they entered the cotton.

In June, 1936, the experiment described above was repeated. However, at the end of 48 hours, because of the higher temperature at this time, some of the seeds had produced seedlings with roots 4 mm. long. Such seedlings were dissected out of the fruit and mounted in aceto-carmine. In June, 1937, seeds of *Setaria viridis* were exposed to infection and the seedlings fixed as described above for *S. italica*.

Observations made on the material prepared by the aceto-carmine method showed that the fungus entered the root or coleorhiza by penetrating directly through the epidermal cells. It was apparent from such observations that the entrance of the germ tube could take place at an early period in its development. Figure 1, A, was made of an oospore that had germinated in contact with the coleorhiza. The growth of the hypha after it had entered the tissue of the host was followed for some time in the material stained with

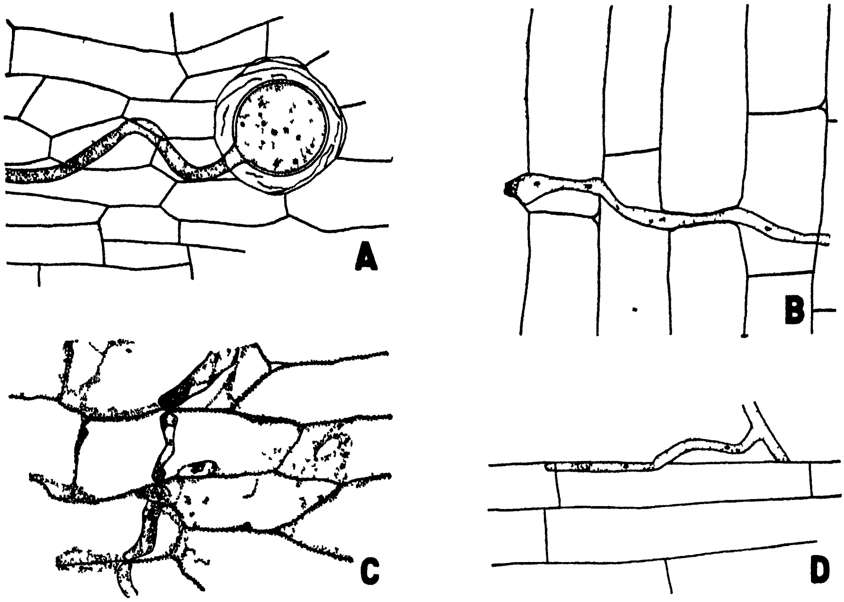

FIG. 1. A. An oospore whose germ tube had penetrated into the coleorhiza of a 24-hour-old *Setaria italica* seedling. ×150. B. Hypha entering root of *S. italica*. ×150. C. Entrance of an infection hypha into the epidermal cell of an *S. italica* root. ×150. D. Intracellular growth of Sclerospora hypha in the coleorhiza of *S. viridis*. ×225.

aceto-carmine, but, because of the thickness of such preparations, it was found impossible to continue accurately the observations of the history of the infection hypha in older seedlings. Therefore, such observations were made on plant material imbedded in paraffin (Fig. 2).

The results of the present investigation indicated that the germ tube had penetrated directly into the seedling through the epidermal cells. Occasionally, as is indicated in figure 1, D, the germ tube was found to branch before it had entered the root of the host. The investigations did not disclose the extent of the growth of the germ tube that occurred between germination and the time the infection. It would seem, however, that this growth was rather limited.

Germ tubes were observed to have entered the coleorhiza, the root, the coleoptile, and the mesocotyl. The larger number of infections was, however, observed to have taken place through the epidermis of the root and the coleorhiza. Germ tube penetration into the very young embryonic region of the root or into the root cap was observed to have taken place only rarely. It is likely that the present method of inoculation did not favor the infection of the tip of the root, or the penetration of the germ tube into the regions of the seedling that, normally, would grow above ground. No infection was observed to take place through root hairs.

After the infection hypha had entered the epidermal cell it was found to penetrate directly through the host cell or to branch within the cell. Further development of the hypha or a branch from the original infection

thread was found to be intracellular until several of the host cells had been penetrated. Eventually, the parasite became intercellular. However, even after the mycelium had penetrated for some distance into the root, hyphae were found, at times, to have passed through the cell walls (Figs. 1, C, and 2, A). Haustoria were found on intercellular mycelium shortly after infection had taken place.

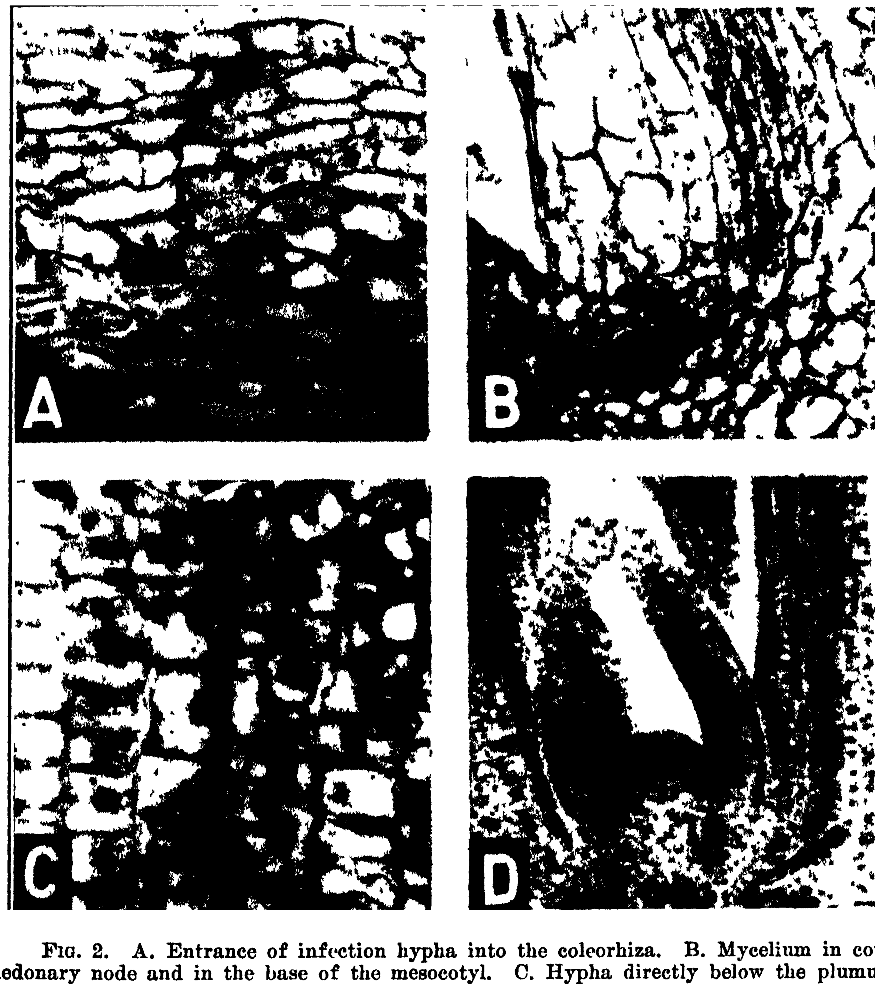

FIG. 2. A. Entrance of infection hypha into the coleorhiza. B. Mycelium in cotyledonary node and in the base of the mesocotyl. C. Hypha directly below the plumule. A haustorium may be seen. D. Mycelium in the embryonic region. A, B, D: ×207. C, ×480. Photomicrographs by F. A. Bautsch, S.J.

In certain instances, after the germ tube had penetrated into a cell, it was observed to have swollen considerably on both sides of the wall at the point of entrance. An interesting example of this is illustrated in figure 1, B, in which it may be seen that the infection hypha, soon after having entered into the epidermal cell of the host, has increased greatly in size at a point next to the outer wall of the host cell. A piece of the wall has been pushed outward under the pressure developed by the hypha.

Sections of plants that had been fixed when the coleorhiza was just emerging from the seed were found, in some instances, to have already become infected. In such cases the infection hyphae of the fungus could be seen to have entered the coleorhiza. Two of 10 seedlings so sectioned at this stage were found infected. In no case was the mycelium observed in sections of embryos of ungerminated seeds that had been exposed to infection. No infection was seen in sections made of the control seedlings nor were conidia observed at any time on any of the control cultures.

Sections of seedlings made 24 hours after they had started to emerge from the seed showed the fungus present in 5 of the 7 seedlings examined. In one instance, the growth of the mycelium of *Sclerospora* was limited to the root; in 2, to the coleorhiza; and in 3, it was found in all parts of the young seedling except the coleoptile.

Roots of seedlings 24 hours old often showed an extensive growth of the fungus. Although it was observed that the fungus spread through the root, the actual amount of mycelium found in the roots was not great, even in plants with mycelium in the embryonic stem. The coleorhiza at times contained a widespread mycelium at an early stage. Some preparations indicated, without doubt, that the initial infection had taken place through the coleorhiza. It was not uncommon, however, to find the root, as well as the coleorhiza, infected at several points. Because of the intimate connection of the coleorhiza with the rest of the seedling, and especially because of the easy access afforded by the cotyledonary node to the parts of the plant aboveground, it seemed that the typical infection point was the coleorhiza.

The cotyledonary (scutellary node) was observed to contain the hyphae of *Sclerospora* in seedlings 24 hours old. This mycelium was found in some instances continuous with that of the root and at other times with that of the coleorhiza.

The cotyledon (scutellum) also became infected by the pathogen. Mycelium was observed ramifying through the cotyledon, but not densely filling it. The hyphae had penetrated as far as the epithelial layer.

It was observed in some cases that the mesocotyl had been entered directly by the pathogen. Under the conditions of these experiments the spread of the pathogen into the mesocotyl was found to occur from the cotyledonary node, which, in turn, had become infected by mycelium from the coleorhiza or the root. It was possible to trace the mycelium in some seedlings from the initial infection in either the root or the coleorhiza to the mesocotyl (Fig. 2, A, B). Once having entered the mesocotyl, the growth of the fungus was directed almost entirely toward the embryonic region of the stem. The path of the mycelium was through either the cortex of the mesocotyl or through the stele. As is shown in figure 2, C, D, the hyphae entered directly into the meristematic region. From this region the fungus infected the young leaves and branches as they were produced (Fig. 2, D).

In 14-day-old plants that were sectioned were found hyphae bearing conidiophores. The hyphae found in the nodes and internodes of such

plants did not stain heavily in the region of the meristem. Such hyphae were apparently inactive at that time.

Plants infected with *Sclerospora* showed a darkening of the primary root, accompanied in some cases by necrosis. Upon examination such roots were found invaded by the mycelium of *Sclerospora* and also that of other fungi. Spores of *Helminthosporium* and *Fusarium* were found in dishes containing seedlings with necrotic roots, and it can be assumed that the septate mycelium observed was that of one of these fungi. Such roots often were observed to contain much mycelium and to rot away completely. On the other hand, apparently healthy roots showed on examination the presence of the mycelium of *Sclerospora*. The roots of young plants that had produced conidia were examined macroscopically and many plants were found with normal roots.

Stunting, caused by a shortening of the mesocotyl and of the internodes, was one of the most noticeable effects of the pathogen on the early development of the host seedlings. Such a dwarfing was often noticeable even in plants only a few days old. However, after 14 days of growth, some seedlings that produced conidia were observed to be as tall as the checks.

Longitudinal sections of dwarfed seedlings, 14 days old, showed marked areas at the top of the mesocotyl, and in the internodes of the plant. Such areas were often located in the meristematic tissues of these regions. At earlier stages it was observed that the mycelium in the plumule caused a local necrosis, apparently brought about by substances directly or indirectly produced by the fungus, since cells not in contact with the pathogen were found to be necrotic. Because of these observations it was thought that the dwarfing might have resulted partly from a necrosis of meristem cells. Further work on this phase of the problem is contemplated.

SUMMARY

Infection of *Setaria italica* and *S. viridis* seedlings by *Sclerospora graminicola* was found to take place through either the root, the coleorhiza, the mesocotyl, or the coleoptile. The germ tube of the organism, or a branch from it, was observed to pass directly through the epidermal cells.

Seedlings were observed infected when the coleorhiza was just emerging from the seed. Since the coleorhiza is usually the first part of the seedling to emerge, the natural entrance of the fungus is considered to be most often through this organ.

The pathogen was observed to have become systemic before the mesocotyl had elongated to any extent and before the coleoptile had penetrated far from the seed.

The early growth of the fungus in the plant was found to be intracellular, but an intercellular mycelium with haustoria was formed after the infection hypha had penetrated a few cells of the host.

After the fungus had entered the plant, growth was directed toward the embryonic stem tip. From this region the mycelium entered the young leaves of the plant as they developed.

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NAUCORIA ON SMALL GRAINS IN ILLINOIS

G. H. BOEWE

(Accepted for publication May 9, 1938)

In the summer of 1935, the writer found a small gill fungus of the genus *Naucoria* growing on wheat plants in Massac County, Illinois. Only a small area in the field, a hillside with west exposure, was found infested; and relatively few plants in the area were attacked. When this collection was made, May 16, most of the plants were headed out. In 1936, the same field was in wheat and again the fungus was found on this host in the same area in the field, but the intensity of attack was less, probably due to the dry weather of that year. Later in the summer of 1935, the same fungus was found on barley and rye in widely separated counties of the State.

Many species of *Naucoria* grow on dead plants and on soil, but very few have been reported as definitely attacking living plants. Indeed, relatively few agarics grow on living herbaceous plants and induce disease. According to Cook (4), however, a toadstool, thought to be parasitic, was found growing on sugar cane in Java, which Wakker described in 1895 as *Marasmius sacchari.* Since then, its parasitic habit has been definitely proved and its occurrence has been established in many places in warmer regions. Other species of *Marasmius* have been reported on various hosts by several workers. Cobb (2) records the occurrence of *Marasmius* on sugar cane in Hawaii, and Fulton (6) and Edgerton (5) state that sugar cane in Louisiana is attacked. According to Cook (4), "The geographical distribution of *M. sacchari* and related species may be summarized as follows,—*M. sacchari* has been reported from Java, India, Australia, Formosa, Hawaii, Puerto Rico, Jamaica, Lesser Antilles, British Guiana and South Africa; *M. plicatus* from Java, Philippines and United States; *M. stenophyllus* from Santo Domingo and Lesser Antilles; *Marasmius* spp. from Fiji, Central America and Brazil; and *Hypochnus sacchari* from Cuba and Jamaica." Cook (3) reports species of *Marasmius* causing disease on sugar cane, tea, cocoa, nutmeg, and banana. In 1922, Young (9) collected a *Marasmius* on wheat in Illinois and later described it as *M. tritici.* It was found on oats, rye, barley, *Agropyron repens* (L.) Beauv., and an unidentified grass. Mains (7) reports finding *Marasmius insititious* Fries on several clumps of *Festuca capillata* Lam. in Indiana.

In 1937, the writer (1) reported the finding in 1935 of his *Naucoria* on wheat, rye, and barley in Illinois, and, in 1938, Sprague (8) reported observing *Pholiota durata* (Bolt.) Fries, *P. praecox* (Pers.) Fries, *Naucoria* spp., and a number of small, delicate, undetermined agarics attacking or associated with crowns of oats, barley, and wheat in western Oregon.

A few species of *Naucoria* are listed in Saccardo's Sylloge Fungorum as occurring on living plants, namely, *N. musarum* Pat. and Demange on stems of mosses, *N. paludosella* Atk. on sphagnum and other mosses, and *N. pityrodes* Briganti on "semi-living" culms of *Agrostis vulgaris* With. Several other species are listed as occurring on stems of various monocotyledons, but it is not clear whether the host was living or dead. One, *N. graminicola* Nees, is reported on stalks and culms of grasses, in Europe.

The description of none of these species fits the fungus found by the writer on wheat, rye, and barley.

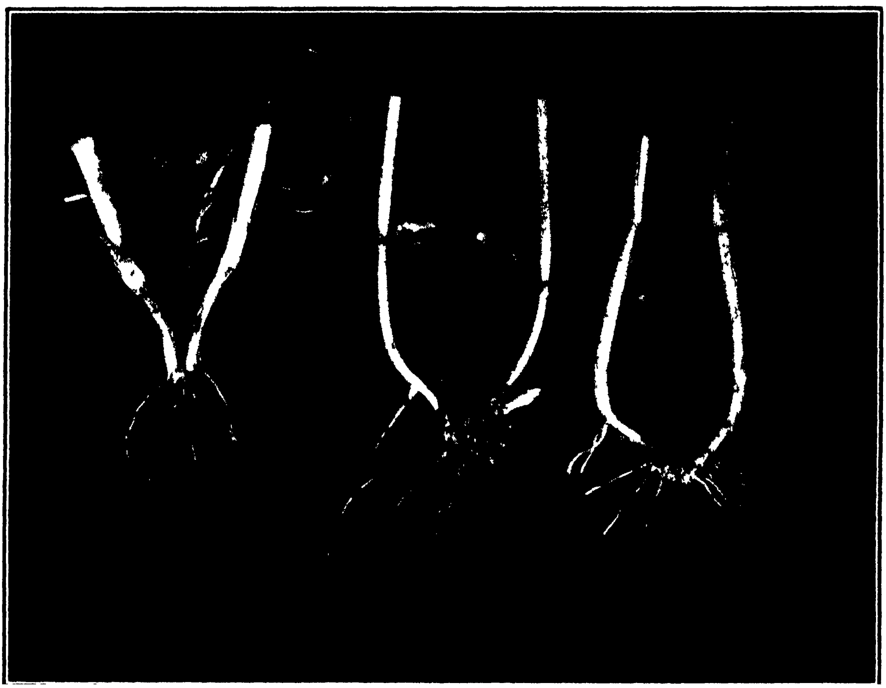

FIG. 1. *Naucoria* fruiting on tillers of living wheat (2 at right) and barley (left), collected in Illinois in 1935.

Most of the sporophores were attached to dead tillers (Fig. 1), but some were attached by white rhizomorphs to roots that were apparently living and functioning. By teasing apart affected tillers and the portion of the crown from which they arose, a whitish cast, due to the fungus mycelium, was observable. Examining microscopically hand sections of affected root tissues, the mycelium of the fungus was found beyond the point of attachment of the rhizomorph. The fact that this toadstool generally was found

fruiting on dead tillers and that its mycelium ramifies through the crown and roots to which it is attached, suggests that it is at least mildly parasitic, an assumption that is supported by the fact that unattacked tillers grow to nearly normal height. In rye and barley the sporophores were attached more often to crowns of the plants than to dead tillers.

The sporophore of this fungus has no ring, the spores are golden brown, the stipe is cartilaginous, the gills are not decurrent, and the margin of the pileus is at first incurved, characters that place it definitely in the genus *Naucoria*. Since it differs from all other species of this genus, the descriptions of which the writer has been able to find, he considers it a new species for which he gives the following diagnosis.

Naucoria cerealis, n. sp.

Pileus thin, membranous at first, hemispheric and then expanded, sometimes with depressed center and margin turned down, 1–2.2 cm. broad, glabrous, light buff, flesh whitish; lamellae adnate, broad, thin, chestnut; spores golden-brown, oval in one view but asymmetrically oval in the other, with a gelatinous apicule covering the germ pore, smooth, 9.9–16.5 × 6.9–9.9 μ, generally 12.5–13.5 × 7–8.5 μ; stipe equal, slightly bulbous at base, glabrous, light buff, stuffed, becoming hollow with age, striate in upper portion, 2–4.6 cm. long, 1–1.5 mm. thick.

Pileo tenui, membranaceo primitus, hemisphaerico dein expanso, nonnumquam cum centro depresso et margine deorsum curvato, glabro, argillaceo, carne albido, 1–2.2 cm. lato; lamellis adnatis, latis, tenuibus, castaneis; sporis aureis-brunneis, hinc ovalibus sed illinc asymmetrice ovalibus, cum apiculo gelatinoso supra poro germinationis, laevibus, 9.9–16.5 × 6.6–9.9 μ, plerumque 12.5–13.5 × 7–8.5 μ; stipite aequali, basi leniter bullate incrassato, glabro, argillaceo, farcto, in aetate cavitato, striato sursum, con pileo concolori, 2–4.6 cm. alto, 1–1.5 mm. crasso.

Fruiting on functioning roots and dead tillers of living wheat and on crowns and tillers of rye and barley.

Type specimen is Accession No. 25602 in the Mycological Collection of the Illinois State Natural History Survey, on wheat, Massac County, Illinois, May 16, 1935. Other collections are: No. 25603 on *Hordeum sativum* Jessen., Cora, Jackson County, June 5, 1935; No. 25413 on *Secale cereale* L. near Chadwick, Carroll County, June 22, 1935; No. 25414 on same host near Georgetown, Vermilion County, June 11, 1935; and No. 25483 on *Triticum vulgare* L., May 12, 1936, from the same field in Massac County as in the previous year.

SUMMARY

The occurrence of a *Naucoria* on wheat, barley, and rye in Illinois is reported. It is believed to be weakly parasitic, and is described as a new species, *N. cerealis*.

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CHLOROTIC STREAK OF SUGAR CANE IN THE UNITED STATES

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(Accepted for publication June 23, 1938)

Chlorotic streak, a disease of sugar cane, until recently (1, 5) unreported in the continental United States, has been observed in Louisiana during the fall of 1937 and the spring of 1938. This disease, possibly of virus origin, is known to occur in Java, Australia, Hawaii, and Puerto Rico.

The symptoms of the disease as observed in Louisiana are identical with those described by Martin (2, 3) and Wilbrink (6). The streaks first appear as faint, narrow, elongate, chlorotic areas, with wavy, irregular margins, later becoming larger and more sharply defined, and frequently developing papery, necrotic centers (Fig. 1). In size they vary from a faint line to $\frac{3}{8}$ inch in width, and in length from a fraction of an inch to almost the entire length of the leaf. The streaks may occur on any part of the leaf blade, but do not extend to the sheath, and may appear on only one or on several leaves of an affected plant.

In order to determine whether the chlorosis was transmitted in seed cuttings, thus affording a more positive identification as chlorotic streak, cuttings of 3 affected varieties were divided into 2 lots, 1 of which was treated with hot water at 52° C. for 20 minutes, which both Wilbrink (6) in Java and Martin (3) in Hawaii found cured the disease, and the other was left untreated as a control. Single-eye pieces from each lot were then planted in steamed soil in 4-inch pots in an insect-proof greenhouse.

All of the plants arising from eyes treated with hot water came up healthy and remained so during a 6-month period of observation. Sixty-four per cent of those from nontreated eyes developed typical symptoms of chlorotic streak that persisted during the course of the experiment. The transmission of the typical symptoms in nontreated cuttings and their elimination by the hot-water treatment were considered definite proof that the disease was chlorotic streak.

When first observed in August, 1937, the disease was found on 4 unre-

leased seedling varieties (C. P. 29/99, 33/229, 33/243, and 33/253) in a test plot on one plantation. It reappeared in the ratoons of these varieties in the spring of 1938, and in plant cane, the seed for which had been cut from the affected stools in the fall of 1937. Several diseased stubbles of the C. P. 29/99 developed only 1 or 2 rather weak shoots, indicating that the disease was causing some suppression of germination.

FIG. 1. Chlorotic streak on mature leaves of the sugar-cane variety C. P. 29/99. Note distinctive, wavy-margin lesions and beginning of papery, necrotic centers in the streak on the leaf at the top.

In a later survey by R. D. Rands and the writer to determine the distribution of the disease, chlorotic streak was found on additional seedling varieties on another plantation in Louisiana, and to the extent of 10 to 15 per cent in fields of the commercial variety C. P. 29/320. It is now known to occur in at least 4 parishes of that State. The disease was not found in the sugar-cane-growing sections of Florida nor in southern Georgia. A further survey of the sugar-cane district of Louisiana is now in progress.

The origin of the disease in the United States has not been determined. National quarantines Numbers 15 and 16 have prohibited importation of sugar cane from foreign countries and insular possessions since June 6, 1914. It is unlikely that this disease could have been introduced with foreign varieties imported by the United States Department of Agriculture, because of the stringent quarantine regulations. Such importations are subjected to several hot-water treatments during the course of a year or more while being propagated under observation in quarantine at Arlington Farm, Virginia,

before distribution to commercial areas. Therefore, except through possible unauthorized or smuggled importations of cuttings since 1914, the disease may have been introduced prior to the institution of the Federal quarantines, and have persisted on sugar cane or some wild-grass host without being detected, possibly on a symptomless carrier. The existence of such has not been demonstrated, and a careful inspection of grasses in the vicinity of the sugar-cane fields where the disease was found has failed to reveal symptoms more than faintly suggesting chlorotic streak.

Although no local attempts have been made to transmit the disease, either by artificial inoculation or by insects, efforts in other countries (4), involving various mechanical methods and such insects as the cane aphid and leaf hopper, the corn aphid, and the onion thrips, have given only negative results.

Of the present commercial varieties, C. P. 29/320 appears to be very susceptible to infection. Other commercial varieties with apparently equal exposure to the disease have contracted it to a much less extent. The information available at present is, however, unsufficient to permit a classification of varieties with respect to susceptibility to the disease. The hot-water treatment offers a means for starting healthy seed plots of infected susceptible canes.

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Relations between Crown Gall and pH of the Soil.—Almost 20 years ago, Dr. M. B. Waite informed the writer that he considered crown gall to be the most serious in regions where the soil is relatively alkaline. Later, when comparisons were made between organisms isolated from various malformations on apple and other hosts the writer[1] called attention to the fact that the organism isolated from "woolly knots" or "hairy roots" of apple exhibited striking differences from the crown-gall organism, *Phytomonas tumefaciens* Smith and Town., in host specificity. A difference in their reaction in litmus milk has frequently been observed. This organism, later classified as *Phyt. rhizogenes* Riker *et al.*,[2] produces growth in media as acid as pH 4.0;[2] the crown-gall organism produces most growth at pH 7,[3] with pH limits at 9.6 to 5.2[4] or 4.4.[2] Accordingly, general observations have been made over a period of years on the prevalence of these two diseases in relation to the pH of the soil. As an example, the situation encountered during an inspection of nurseries growing peach trees in the Willamette Valley, Oregon, and in the Sacramento River Valley, California, may be cited. The soils in the Willamette Valley are relatively acid, and liming is practiced for certain crops; in the nursery in this section almost no crown gall was found. In the nurseries in the Sacramento River Valley, where the soil in general is relatively alkaline, crown gall infections were abundant.

In order to test the hypothesis that relatively alkaline soils may be a predisposing factor for crown-gall infections, 4 adjacent rows, approximately 75 feet long, were selected for planting peach seed. The soil was known to be acid. Two alternate rows were limed in the fall of 1937, and, 1 week later, all 4 rows were inoculated heavily with a water suspension of the crown-gall organism. The seedlings were dug in the fall of 1938 and composite samples

TABLE 1.—*Comparison of the amount of crown gall on peach seedlings grown in[1] relatively acid and in[2] approximately neutral soils*

Row	Treatment	Number of trees	Number of galled trees	Galled trees (per cent)	pH of soil
1	Limed	360	115	32	6.8
2	Not limed	337	12	4	5.0
3	Limed	452	147	33	6.8
4	Not limed	341	6	2	5.0
Total	Limed	812	262	32	6.8
"	Not limed	678	18	3	5.0

1 Siegler, E. A. Studies on the etiology of apple crown gall. Jour. Agr. Res. [U.S.] 37: 301–313. 1928.

2 Riker, A. J., Banfield, W. M., Wright, W. H., Keitt, G. W. and Sagen, H. E. Studies on infectious hairy-root of nursery apple trees. Jour. Agr. Res. [U.S.] 41: 507–540. 1930.

3 Patel, M. K. Biological studies of *Pseudomonas tumefaciens* Smith and Town., and fifteen related non-pathogenic organisms. Iowa State College Jour. Sci. 3: 271–298. 1929.

4 Personally communicated by Agnes J. Quirk.

of soil from each of the 4 rows were taken for pH determinations. The results are given in table 1.

The results show that 32 per cent of the seedlings grown in the limed rows were galled and that 3 per cent of the trees grown in the relatively acid rows were galled. It is highly desirable to confirm these results with similar experiments. Until more data are obtained, however, it is thought that caution should be exercised before advising nurserymen to lime their soils where crown gall is an important factor. It is possible that adjustment of the pH of certain soils may likewise prove to be an aid in controlling hairy root.—E. A. SIEGLER, U. S. Horticultural Station, Beltsville, Maryland.

Spine Development on the Spores of Ustilago zeae.—Ustilago spore formation was first noted in detail by de Bary in 1853; some additional contributions, especially with regard to the cytology were made by Lutman and Seyfert. The mycelium forms jelly-like nests composed of hyphae in worm-like coils among the corn cells. At maturity, the hyphae break up into short, binucleate bodies surrounded by a thick wall of jelly. In the final stage the thick, jelly-like wall becomes brown and covered with spines.

The development of a spiny wall from a gelatinous matrix is somewhat puzzling and seems not to have been studied, perhaps because of the small size of the smut spores. A permanent stain was sought that would bring out the gelatinous layer, the developing wall with its spines, and at the same time reveal, as much as possible, the spore contents. Hutchinson's stain combination[1] of orseilline BB (Grübler) with aniline blue, used in staining freshly-cut sections of white pine for the blister-rust mycelium, was among the combinations tested. He found "mycelium stained violet to blue, suberized and lignified walls are red. Haustoria . . . stain a deep red in contrast to the bluish mycelium outside the cell wall." This combination was Strasburger's adaptation of Mangin's discovery of the value of orseilline (a dye obtained from various lichens) as a test for cellulose. The technique used for staining the sections of corn bolls infected with *Ustilago zeae* was different in a number of steps from that used by Hutchinson.

Sections from material fixed in the Allen-Bouin mixture were transferred to water and then stained in a saturated orseilline BB solution, about 1 gram of orseilline in 30 cc. of 3 per cent acetic acid, for 24 hours. 50 per cent alcohol was then run quickly over the slide to remove the excess stain, and the sections were then placed in a saturated solution (1 g. of aniline blue in 100 cc. of 3 per cent acetic acid) for 24 hours. The sections were dehydrated quickly by flushing with absolute alcohol, immersed in xylol, and mounted in balsam.

This clear, differential stain brought out the various stages in the development of the smut-spore walls. A sphere of dark-red material (exospore) formed around the cytoplasmic material, inside the blue-staining,

[1] Hutchinson, W. G. A method for staining rust mycelium in woody tissues. Phytopath. 26: 293–294. 1936.

gelatinous covering of the spore *anlage*. From this sphere, minute brilliant red spines protruded out into the blue-stained jelly of the primitive walls. In shape these spines varied from structures with sharp points to those tipped with small knobs. Many were cone-shape, with considerable variation in length and thickness. In the meantime, the spore itself was growing rapidly in bulk and rounding up; the mature spores with spines being at least 50 per cent larger than the irregular, blue-walled *anlage* derived from the mycelium. In later stages, the blue jelly, which once surrounded the spines and in which they were imbedded, appeared to collapse and disappear. The spines, which stained originally a brilliant red from the orseilline, indicating their lignified nature, changed to a gray, and finally to a brown color, denoting the chemical stages that occurred as they grew and became impregnated with chemicals other than cellulose or lignin (Fig. 1.).

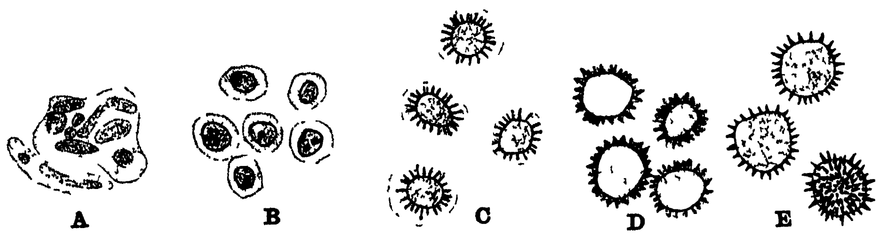

FIG. 1. Spine formation on the spores of *Ustilago zeae*. A. Fragmentation and the rounding up of mycelium with thick, blue, jelly-like walls. B. Inner wall thickens, stains red, as does protoplasmic content. C. Young, red spines protrude from the dark red wall into the blue jelly, which later (D) collapses over the spines. E. A mature spore with gray-brown spines results. Series is magnified about 1000 ×.

Sappin-Trouffy,[2] using methyl blue and either Grenacher's or Boehmer's hematoxylin, but with no details as to methods, was so much fascinated by the binucleate-cell condition in the rusts that he found very little to say about spore-wall development. His accurate drawings, however, indicate clearly that he saw a similar spine formation in the uredio-, aecio- and teliospores as has been just described for smut spores. Wall and spine development are very much easier to follow in the rusts than in the smuts, since the former are so much larger; but the two processes are undoubtedly analagous.

The orseilline BB and aniline blue stain deserves a revival by plant pathologists and mycologists if it is as successful on other fungi as it has proved on smuts and rusts. The majority of the blue stains that the writers and others have tried are very evanescent, but, after 4 months, the sections stained with this combination are as brilliant blue and red as they were the day they were made.—H. L. HUTCHINS and B. F. LUTMAN, University of Vermont, Burlington, Vermont.

The Formation of Chlamydospores of Ustilago crameri Kcke. on Artificial Media.—Numerous chlamydospores were produced in single-chlamydo-

[2] Sappin-Trouffy, P. Recherches histologiques sur la famille des Urédinees. Le Botaniste 5: 59–244. 1896.

spore cultures of *Ustilago crameri* Kcke., grown at room temperature for about ten weeks on 1, 2, and 3 per cent potato-dextrose or malt agar in flasks and tubes. Spores were produced equally well on either potato-dextrose or malt agar, but, in general, the cultures produced chlamydospores quicker on the more concentrated media than on the dilute media. These results were obtained in repeated tests and partially corroborate the work of Sartoris[1] with other smuts.

The chlamydospores are formed in an intercalary manner, either from the hyphal strands or from short segments formed by fragmentation of the mycelium, the latter condition occurring infrequently. At the beginning of chlamydospore formation, the mycelial cells, or occasionally alternate cells only, enlarge and become surrounded by a gelatinous membrane. As the spore enlarges still further, the gelatinous membrane apparently is broken and many of the cells disintegrate. There is no wall other than the gelatinous membrane until the spores are two-thirds or more of the mature size. After this a normal episporium and endosporium are formed, the color of the spore gradually darkens, and the gelatinous membrane disappears.

Permanent slides of stained mycelium were made of the cultures in which chlamydospores were forming. Most of the hyphal cells were binucleate, although uninucleate cells were not uncommon, and an occasional multinucleate one could be seen. During gelatinization most of the cells became uninucleate, and fusion of 2 nuclei was observed commonly at this stage.

The chlamydospores produced as described on sterile media are of the same size and structure as those produced in the host plant, and they germinate in a perfectly normal manner.

Several other smut fungi have been reported to produce chlamydospores on artificial media: Among the cereal smuts, *Ustilago tritici*[1] *U. avenae* (see Wang[2]), *U. hordei* (see Kniep[3]), *U. levis*[2], and *U. nuda*[4]; among other smuts *U. tragopogonis* and *U. scorzonorae*[5], *Entyloma ranunculi* and *E. calendulae*[6], *Urocystis anemones*[2], and *U. gladioli*[7].—C. S. Wang, formerly graduate student, Department of Plant Pathology, University of Minnesota; now, College of Agriculture, Honan University, Honan, China.

1 Sartoris, G. B. Studies in the life history and physiology of certain smuts. Amer. Jour. Bot. 11: 617–647. 1924.

2 Wang, D. T. Contribution à l'étude des Ustilaginées (Cytologie du parasite et pathologie de la cellule hôte). Le Botaniste 26: 539–670. 1934.

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5 Koudelka, H. Neue Probleme in der Brandpilzfrage. Nachr. ü. Schädlingsbekämpf. 9: 100–104. 1934. [Abstract in Rev. Appl. Myc. 13: 749. 1934].

6 Stempell, K. L. Studien über die Entwicklungegeschichte einiger Entyloma-Arten and über die systematische Stellung der Familie der Sporobolomycetes. Zeitschr. Bot. 28: 225–259. 1935.

7 Wernham, C. C. Chlamydospore production on artificial media by *Urocystis gladioli*. Phytopath. 28: 598–600. 1938.

NOTICE TO MEMBERS

Your assistance is again sought in an effort to bring the advantages of membership in our Society to certain others, not yet enrolled, who have an interest in plant diseases. All persons interested in the study of Phytopathology including the practical control of plant diseases shall be eligible to membership.

In addition to active research workers and advanced students such "suscepts" may be found among certain extension pathologists, college and high school teachers, nematologists, entomologists, plant breeders, horticulturists, agronomists, foresters, chemists, officials engaged in plant-disease-control campaigns, in inspection of shipped products, in plant-quarantine enforcement, and in inspection and certification of nursery stock, plants, seeds, etc., county agents, competent persons engaged in manufacture, testing, and sale of fungicides or machinery, and qualified persons engaged in commercial plant-disease control.

It is not the intention that a "high-pressure drive" should be made resulting in the enrollment of a number of people who have no permanent interest in our society, who would soon drop out, and who might do the Society more harm than good. It is, however, the earnest hope that all who would make acceptable members be approached and given a personal invitation to join. Younger men often feel, and rightly, that membership in The American Phytopathological Society indicates an acknowledgment of recognized interest and standing in plant-disease work.

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PROPERTIES OF THE POTATO YELLOW-DWARF VIRUS[1]

L. M. BLACK

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INTRODUCTION

The potato yellow-dwarf virus, carried by the clover leaf hopper, *Aceratagallia sanguinolenta* Prov. (1, 2), is difficult to transmit mechanically. Muncie (9) failed to obtain infections by injection of juice from diseased leaves into healthy tubers. The writer, in preliminary experiments with the carborundum (11) and pin-puncture methods, succeeded in transmitting the virus to only an occasional plant. Further investigation of the problem has led to the discovery of a host (*Nicotiana rustica* L.) to which the virus can be transmitted with ease. The use of this species has made it possible to determine readily some properties of the virus that otherwise would have been extremely difficult or impossible to study. The following paper is an expanded account of results briefly reported elsewhere (3).

NEW HOSTS OF THE VIRUS

In the course of the investigation it was found that the yellow-dwarf virus could be transmitted to a number of plants not hitherto demonstrated to be susceptible. The new hosts listed in table 1 were inoculated by either infective leaf hoppers or by grafting. In every case the virus was transferred from the new host back to a plant upon which it produced known distinctive symptoms. At first, Green Mountain potatoes (*Solanum tuberosum* L.) were used for this purpose. Later it was found that the virus had a shorter incubation period in *Nicotiana glutinosa* L. and the latter was substituted as a test plant.

SEPARATION OF THE YELLOW-DWARF VIRUS FROM THE X-VIRUS

When potato plants were employed as a source of yellow-dwarf virus for the inoculation of other plants, symptoms were sometimes attributable, in part at least, to the X-virus, which is usually present in commercial potato varieties. A pure source of yellow-dwarf virus was indispensable for experimental purposes. In preliminary experiments it had been found that the two viruses, when introduced into *Nicotiana glutinosa* by scions from infected potatoes, produced a severe disease. To obtain the yellow-dwarf virus free from the X-virus, *N. glutinosa* plants were inoculated by means of infective leaf hoppers taken from clover. Some of the plants developed pronounced and distinctive symptoms. These plants were shown to contain the yellow-dwarf virus (Table 1) and to be free of the X-virus. Their juice failed to infect 100 Ruby King pepper plants, whereas control

[1] This study was conducted at The Rockefeller Institute for Medical Research, Princeton, N. J., during the tenure of a National Research Council Fellowship in the Biological Sciences.

TABLE 1.—*Transmission of the potato yellow-dwarf virus from new hosts to suitable test plants*

New host	Test plant to which virus transmitted	Transmitted by means of	Infections on[a]		Remarks on new host
			Test plants	Control plants	
Nicotiana glutinosa L.	*Solanum tuberosum* L. var. Green Mountain	Grafts	12/20	0/20	Distinctive symptoms
Nicotiana langsdorffii Weinm.	"	"	5/5	0/5	
Nicotiana sylvestris Spegaz. and Comes	"	"	7/10	0/10	
Callistephus chinensis Nees. var. Semple's Shell Pink	"	*Aceratagallia sanguinolenta*	7/8	0/8	Very mild symptoms
Trifolium incarnatum L.	"	"	5/5	0/5	Severe symptoms in acute stage
Vicia faba L.	"	"	8/10	0/10	
Nicotiana rustica L.		"	2/5	0/5	
(inoculated by rubbing)	"	Grafts	2/5	0/5	
Nicotiana rustica L. (inoculated by grafts)	*Nicotiana glutinosa* L.	"	7/12	0/12	
Nicotiana glauca R. Grah.	"	"	8/10	0/10	Leaves abscissed
Nicotiana paniculata L.	"	"	9/10	0/10	
Nicotiana sanderae Sander	"	"	9/10	0/10	
Nicotiana tabacum L. (Turkish)	"	"	13/20	0/20	Symptoms mild
Physalis pubescens L.	"	"	4/10	0/5	
Solanum melongena L. var. Black Beauty	"	"	10/10	0/10	

[a] The denominator represents the number of plants used, the numerator the number infected. When leaf hoppers were employed, the controls were treated exactly like the test plants except that the leaf hoppers placed on them had been fed on healthy plants of the new host instead of diseased ones.

pepper plants, inoculated with X-virus, gave 100 per cent infection. Serological tests for X-virus conducted by K. S. Chester were also negative.

The conspicuous symptoms of yellow dwarf on *Nicotiana glutinosa* (Fig. 1) suggested that this species might be a good source of inoculum. Accordingly, it was employed as a source of virus in a search for a host that would develop primary or systemic symptoms when dusted with carborundum and then rubbed with a cheesecloth pad soaked in virus-containing juice. Entirely negative results were secured in tests of 54 different species and varieties of plants. In tests with an unidentified variety of *N. rustica* L.,[2] however, yellow primary lesions (Fig. 2) developed 7–9 days after inoculation. These enlarged from their original size of about 1 mm. in diameter to a final size of about 10 mm. in diameter.

[2] An unidentified variety herein referred to as *Nicotiana rustica*.

FIG. 1. Characteristic systemic symptoms on *Nicotiana glutinosa* invaded by the potato yellow-dwarf virus. Photograph by J. A. Carlile.

MECHANICAL TRANSMISSION IN NICOTIANA RUSTICA

When the virus was transferred from *Nicotiana rustica* to *N. rustica,* much larger numbers of primary lesions were obtained than when *N. glutinosa* was used as a source. As many as 1350 lesions were counted on a single half-leaf. Sometimes a leaf bore so many lesions that it turned completely yellow a few days after the spots first appeared. Moreover, primary lesions can be obtained on *N. rustica* by rubbing the leaves without the use of carborundum. The 5 other varieties of *N. rustica* (English, Iowa, *jamaicensis, pumila,* and Winnebago) that have been tested, all developed primary lesions as a result of inoculation with yellow-dwarf virus. It is also interesting that *N. paniculata* L. and *N. undulata* Ruiz. and Pav. (5), the descendants of the probable parental species of *N. rustica,* both develop primary lesions when inoculated with the yellow-dwarf virus.

Since the work of Holmes (6), several viruses have been described as causing primary lesions on certain hosts. In the present case the growth of the lesions over a period of 2 or 3 weeks after their appearance and the subsequent development of systemic symptoms of yellow dwarf in the new growth leave little doubt as to the agent responsible for the spots. The virus from such plants has been returned to Green Mountain potatoes both

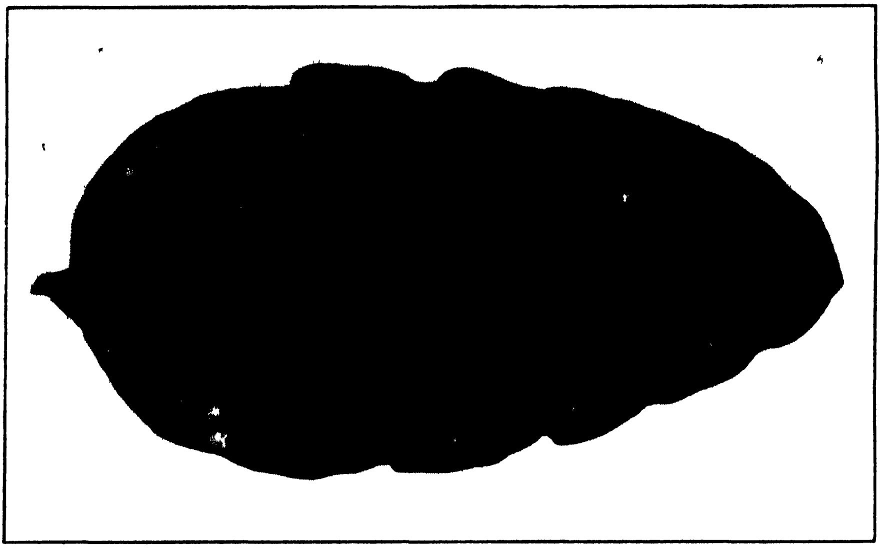

FIG 2. Primary lesions produced by the potato yellow dwarf virus on a leaf of *Nicotiana rustica*. Photograph by J. A. Carlile.

by grafting and by insects (Table 1), and has resulted in typical yellow dwarf.

MECHANICAL TRANSMISSION TO OTHER HOSTS

The carborundum method of transmitting the potato yellow-dwarf virus on *Nicotiana rustica* was compared with that on potato (*Solanum tuberosum* var. *Green Mountain*), *N. glutinosa*, Turkish tobacco (*N. tabacum* L.), *Hyoscyamus niger* L., and crimson clover (*Trifolium incarnatum* L.) In the first part of the experiment, juice from diseased plants of the host in question was used to inoculate healthy plants of the same species and also to inoculate *N. rustica*. From the results (Table 2), it is apparent that

TABLE 2.—*Comparison of Nicotiana rustica with other species as test plants for potato yellow dwarf virus*

Source of the virus	Inoculation of the same species		Inoculation of *N. rustica*	
	Number of plants inoculated	Number infected	Number of half leaves inoculated	Number of primary lesions
Solanum tuberosum L. var. Green Mountain	11	0	5	281
Nicotiana glutinosa L.	10	2	32	345
Nicotiana tabacum L. var. Turkish	20	1	5	13
Hyoscyamus niger L.	10	0	5	26
Trifolium incarnatum L.	26	0	9	482

N. rustica proved more susceptible to inoculation by the juices of these plants than did the source plants themselves. In the second part of the

experiment, juice from diseased *N. rustica* plants was used to inoculate the other host species. The results (Table 3) show that in the 3 cases in which transmission was secured the *N. rustica* juice was superior to the juice from the specific host plant in producing infections. In the case of *N. glutinosa,* apparently the most susceptible of the 5 species tested, about 5 primary lesions were produced on each inoculated leaf when *N. rustica* was used as the source of the virus. No primary lesions were observed when this species was inoculated with juice from diseased plants of the same species. These results are probably attributable to the fact that the virus reached a higher concentration in *N. rustica* than in the other species.

TABLE 3.—*Infections obtained in different species when inoculated with yellow-dwarf virus from Nicotiana rustica and other hosts*

Species inoculated	Source of inoculum			
	The same species		*Nicotiana rustica*	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Solanum tuberosum L. var. Green Mountain	11	0	11	0
Nicotiana glutinosa L.	10	2	19	19
Nicotiana tabacum L. var. Turkish	20	1	20	10
Hyoscyamus niger L.	10	0	10	10
Trifolium incarnatum L.	26	0	15	0

Extensive experiments were undertaken in an attempt to find a successful means of mechanical transmission from potato to potato and from clover to clover. Various methods of transmission from diseased to healthy Green Mountain potatoes were tried. Using carborundum of various grades, juice from yellowing leaves of plants in the acute stage of the disease was rubbed on the upper and lower surfaces of the leaves of young healthy plants. It seemed that transmission was difficult because of the possible rapid inactivation of the virus by oxidation. Accordingly, potassium cyanide, which inactivates aerobic oxidases and peroxidases, and sodium sulphite, a reducing agent, were tested separately for a possible beneficial action. Some inoculations were made in an atmosphere of carbon dioxide. None of these methods increased the number of infections obtained. In other experiments pins of various diameters were thrust through diseased leaves or into juice from such leaves and then into terminal buds, axilliary buds, stem tips, leaves, the eyes of the tubers, and the vascular ring of small seed pieces. Juice alone and juice mixed with carborundum were injected with a hypodermic needle into the stems and tubers. Carborundum and juice were rubbed into the freshly exposed surfaces of the split stems of young plants. The plants were observed from time to time for 4–6 weeks after inoculation. Of 980 inoculated plants surviving these treatments, 104 (10.6 per cent) developed yellow dwarf, while out of 179 noninoculated control plants none developed the disease. Most of the inoculation methods could be classified as modifications of

either the pin-puncture or the carborundum method. Of 237 plants inoculated by some modification of the carborundum method, only 6 (2.5 per cent) became diseased. On the other hand, of 670 plants inoculated by some modification of the pin-puncture method, 92 (13.7 per cent) became diseased. The best transmission obtained in any experiment was only 50 per cent. This was secured by placing juice from leaves in the acute stage of the disease around the eye of an unsprouted seed piece and making about 100 punctures with ordinary pins through the juice into the flesh of the tuber.

The symptoms of potato yellow dwarf on crimson clover are quite characteristic. Infected plants first show a pronounced clearing of the veins, which is soon followed by yellowing of the leaves. The plant is considerably dwarfed and may die. However, if it survives the acute stage of the disease, it gradually recovers and eventually appears to be healthy. Because crimson clover has proved to be the most favorable plant on which to study the insect transmission of the virus, the possibility of mechanical transmission to this host was investigated further. In the first experiment (Table 4), juice from diseased crimson clover plants in the acute stage was rubbed over the leaves of 866 plants, using carborundum as an abrasive. In addition, 98 plants were similarly inoculated with juice from infected *Nicotiana rustica* plants. The plants were 2–6 weeks old and were distributed in 5 groups, according to age. Except for the plants in the first age group, which had only one leaf at the time of inoculation, two of the compound leaves on each plant were inoculated by rubbing them 3 times. Six weeks later none of the plants showed definite symptoms of yellow dwarf. Thirty plants that appeared as though they might have early symptoms were held an additional 6 weeks. Only one of these subsequently developed yellow dwarf. This plant may have been accidently inoculated by insects, but this is considered improbable. Juice from each yellow-dwarf crimson-clover plant used as a source of virus was tested on a small interveinal area of an *N. rustica* leaf (an area about 1/10 of the leaf surface). On the average, the 36 plants tested produced 44 lesions per interveinal area, indicating that the failure to transmit was not because of any lack of virus. As shown in table 3, the same method of inoculation was successful in transmitting pea virus 2 (10) and red-clover mosaic virus (15). Comparable plants also were readily infected with yellow-dwarf virus by means of insects. The failure of the carborundum method was confirmed in a second experiment in which 158 inoculated plants were observed for 12 weeks after inoculation. In an accompanying experiment (No. 3), the infectivity of the inoculum was demonstrated by the inoculation of an additional 47 plants with 50 pin punctures through juice from diseased clover into the crown of the plant. Ten plants developed yellow dwarf, while none of the 47 control plants became diseased. It is interesting that the carborundum method, so successful for the transmission of the virus to *N. rustica,* should be so inefficient in transmitting it to potatoes and to clover.

TABLE 4.—*Transmission of 3 viruses from clover to clover*

Experiment	Virus	Source of virus	Method of inoculation	Age of plants in weeks	Leaves inoculated per plant	Plants inoculated	Plants infected	Incubation period in weeks
1	Potato yellow dwarf	Crimson clover	Carborundum	2	1	155	0	
	"	"	"	3	2	182	0	
	"	"	"	4	2	190	0	
	"	"	"	5	2	185	0	
	"	"	"	6	2	154	1	7
	"	*Nicotiana rustica*	"	2–6	2	98	0	
	Pea virus 2	Crimson clover	"	2–6	2	25	21	1–2
	Red clover mosaic	"	"	2–6	2	25	24	1–2
	Potato yellow dwarf	"	Insect	2–6		10	9	3–6
2	Potato yellow dwarf	"	Carborundum	6	7	158	0	
	"		Noninoculated	6		158	0	
3	Potato yellow dwarf	"	Pin puncture	6		47	10	3–5
	"		Noninoculated	6		47	0	

PROPERTIES OF THE VIRUS

The primary-lesion reaction on *Nicotiana rustica* was applied in a study of some of the properties of the virus. Experience has shown that much better results are obtained with this reaction during the winter months than during the summer. It was soon discovered that the age of the *N. rustica* plant and the age of the leaf were important factors in determining the number of lesions. Plants 7–9 weeks old with stems that had not yet rapidly elongated appeared to be most suitable. On such plants the youngest expanded leaf and the next oldest leaf were selected for inoculation. The growing point was removed, the leaves were dusted with No. 320 carborundum, rubbed three times with a cheesecloth pad soaked in inoculum, and washed with tap water. The plants were grown in a greenhouse held at 80° F. or higher, and the primary lesions were counted after about 2 weeks. A sample of juice was applied to just half a leaf on any one plant. Thus, 4 samples could be applied to each plant. Except for the first 2 experiments on thermal inactivation, each experiment was designed so that 4 samples to be compared were applied on every plant and in every leaf position the same number of times (14). In the experiments on dilution and thermal inactivation, where 7 samples were compared, the test plants were divided into 2 equal groups. In one group the first 4 samples were tested and in the other group the last 4 samples were tested, sample No. 4 being tested in each group as a check on the uniformity of the plants. In these cases the total number of lesions obtained for sample 4 was divided by 2 before being incorporated in the tables.

Longevity in vitro at Room Temperature.—Preliminary experiments indicated that the virus survives only a few hours in nontreated juice held at room temperature (23–27° C.). In 2 experiments (Table 5), in which the activity

TABLE 5.—*Total number of primary lesions produced on half-leaves of Nicotiana rustica after holding juice from diseased N. rustica plants at room temperature for various intervals*

Experiment	Number of half-leaves	Interval					
		0 hours	1 hour	2½ hours	4 hours	8 hours	12 hours
1	20	4859	2592	6	2		
2	20	2055	209	18	0		
3	16	10236	.		2558	154	5
4	16	3732			35	0	0

of extracts of yellow-dwarf *Nicotiana rustica* leaves was tested 1, 2½, and 4 hours after extraction, the results indicated that the virus was almost completely inactivated after 2½ hours. However, it became apparent in subsequent experiments designed for other purposes that this was not always so, and in 1 of 2 later experiments (3 and 4) it was shown that the virus survived as long as 12 hours.

An experiment was conducted to determine whether this rapid loss of

infectivity was due to the destruction of the virus or to the toxic effect of old juice upon the test plant. Juice from diseased and from healthy *Nicotiana rustica* plants, respectively, was held at room temperature for either 13 hours or for 0 hours. Four solutions were then prepared as follows and tested immediately on *N. rustica.*

Solution 1. 9 cc. of juice from diseased plants held 13 hours + 1 cc. of such juice held 0 hours.

Solution 2. 9 cc. of juice from healthy plants held 0 hours + 1 cc. of juice from diseased plants held 0 hours.

Solution 3. 10 cc. of juice from healthy plants held 0 hours.

Solution 4. 9 cc. of juice from diseased plants held 13 hours + 1 cc. of juice from healthy plants held 0 hours.

When tested on 16 half-leaves, solutions 1 and 2 produced lesions (120 and 234, respectively), but solutions 3 and 4 did not. This shows that the loss of infectivity within 13 hours is not due to the formation of a substance that interferes with the entrance of the virus into the plant, but is due to destruction of the virus. The experiments on longevity indicate that this destruction is very rapid.

Longevity of Virus in Dried Leaves.—Leaves from yellow-dwarf *Nicotiana rustica* plants in the acute stage of the disease were allowed to dry in the laboratory for 1, 2, 4, 12, and 16 weeks. The various samples were then ground up separately in a small volume of 0.1 M neutral phosphate buffer. Each extract so obtained was tested on 10 half-leaves of *N. rustica.* None gave any lesions.

Dilution.—Dilution experiments have been performed using both phosphate buffer at pH 7.0 and healthy *Nicotiana rustica* juice as the diluents. Dilutions up to 10^{5} were tested, and one sample of the diluent alone was included in each experiment as a control. None of these control samples produced any lesions and they are omitted from table 6, which gives the results of the dilution tests. In 3 experiments no lesions were obtained at dilutions higher than 10^{3}. In one experiment, 1 infection was obtained at a dilution of 10^{-5}. Because of the large number of primary lesions obtained in some other experiments where undiluted juice was employed, it would not be surprising if infections were obtained at even higher dilutions under the most favorable circumstances for inoculation.

TABLE 6.—*Primary lesions produced on half-leaves of Nicotiana rustica with various dilutions of yellow-dwarf N. rustica juice*

Experiment	Number of half-leaves used	Diluent	Total number of lesions produced at a dilution of					
			10^{-5}	10^{4}	10^{3}	10^{2}	10^{1}	10^{0}
1	8	Healthy juice	0	0	9	85	474	634
2	4	Buffer		0	1	8	141	871
3	8	"	1	10	118	996	1421	1952
4	8	"	0	0	2	90	693	100

Thermal Inactivation.—Because of the rapid inactivation of the potato yellow-dwarf virus *in vitro* at room temperature, a single stock solution of juice could not be employed as a source of samples for the various temperature treatments in an experiment. Instead, a large number of leaves were cut up into small pieces about 6 square centimeters in area and the pieces thoroughly mixed. The chopped leaves were held under moist paper towels. A small sample was withdrawn for each temperature determination, ground in a mortar, and about 5 cc. of juice squeezed out through 2 layers of gauze. Samples of this juice were pipetted into small test tubes (7 x 1 cm.) and immersed for 10 minutes in a water bath at the desired temperature. Upon withdrawal, the tubes were cooled rapidly by placing them in cold water, and the treated juice was applied immediately to half-leaves of *Nicotiana rustica.* In experiments 1 and 2 (Table 7) only 1 leaf was used on each test plant, half of the leaf being used to test the sample and the other half being left noninoculated as a check. In experiments 3 and 4, the samples were compared in the manner previously described. The results indicate a thermal inactivation temperature of about 50° C.

TABLE 7.—*Number of primary lesions produced on half leaves of Nicotiana rustica inoculated with samples of yellow-dwarf virus heated for 10 minutes at various temperatures*

Experiment	Half-leaves used	Total number of lesions produced after inoculation with juice heated to the following temperatures (°C.)									
		56°	54°	52°	50°	49°	48°	47°	46°	45°	25°
1	2				3		7	10	29	113	
2	2	0	0	0	0		8		0		1524
3	8	0	0	0	0		0		2031		4923
4	8				0	13	25	28	109	133	2532

Filtration.—Two hundred grams of yellow-dwarf *Nicotiana rustica* leaves were ground with 8 gm. of K_2HPO_4 in a large mortar. The juice was squeezed through 4 layers of cheesecloth and filtered through celite (Standard Super-cel). Two cc. of a 48-hour culture of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* were added to the celite filtrate and the mixture was then passed through a Berkefeld W filter under a pressure of 1 atmosphere. A 1-cc. sample of the celite filtrate to which the bacteria had been added was placed in each of 2 test tubes of nutrient broth. The Berkefeld filtrate was similarly tested in 2 more tubes. Samples of the juice, celite filtrate, and Berkefeld filtrate showed a pH of 7.1 when tested by the glass electrode method. The 3 solutions, undiluted and diluted 1:40 in phosphate buffer at pH 7.1, were tested on *N. rustica* half-leaves. Control inoculations were made with the sterile buffer. So many lesions were obtained in the case of the undiluted samples of juice and the 2 filtrates that lesions were counted only on a disc 2 cm. in diameter cut from the middle of each half-leaf. In the other cases all lesions were counted. The test tubes of broth to which samples of the Berkefeld filtrate had been added did not show any growth after 2

weeks, whereas the test tubes containing the celite filtrate developed a dense growth within 2 days. These results, demonstrating the filterable nature of the virus (Table 8), were confirmed in a second experiment.

TABLE 8.—*Total number of primary lesions produced on half-leaves of Nicotiana rustica after various filtration treatments of juice from diseased N. rustica plants*

Experiment	Dilution used	Half-leaves inoculated	Total number of lesions on half-leaves inoculated with			
			Berkefeld filtrate	Celite filtrate	Unfiltered juice	Phosphate buffer
1	None	8	221[a]	442[a]	540[a]	0
	1: 40	8	65	52	11	0
2	None	4	1203	1306	1200	0

[a] Lesions were so numerous on these half-leaves that they were counted only on a small disc cut from the center of each half-leaf with a cork-borer.

DISCUSSION

The potato yellow-dwarf virus is carried by the clover leaf hopper (1, 2). In experiments previously reported (2), the writer failed to obtain transmission by the common aphids occurring on potatoes and considered that the ability of aphids to transmit the virus (8, 9) was doubtful. With the exception of this virus, Johnson and Hoggan (7) classify all the leaf-hopper-borne viruses in one group characterized in part by the difficulty with which they are transmitted mechanically. In the group, the only viruses that have been so transmitted are those of curly top of beets, with which Dana (4) obtained 50 per cent transmission from plant to plant by pin puncture, and the virus of maize streak, which Storey (12) transmitted from insect to insect. Oddly enough, Storey (13) has been unable to transmit this virus mechanically from plant to plant.

As shown in the present study, the mechanical transmission of the potato yellow-dwarf virus to potato and to crimson clover is comparable with that of the curly-top virus on beets, as reported by Dana. On both these hosts pin punctures into a region where the vascular bundles were aggregated was superior to the carborundum method of leaf inoculation. This may indicate that on these plants the main factor for successful inoculation is the introduction of the virus into the phloem. On *Nicotiana rustica*, however, the success of the carborundum method in producing primary lesions suggests that in this plant the entrance of the virus into the phloem is not necessary and indicates the importance of the host plant in the mechanical transfer of the virus.

Where the properties of other leaf-hopper-borne viruses have been investigated, the technique has involved the laborious process of first introducing the virus into the insect vector and then testing the insect on susceptible plants. The greater ease with which the yellow-dwarf virus has been studied on *Nicotiana rustica* furnishes a striking contrast. Unless this virus is unique among those carried by leaf hoppers, one may expect that some

of the other viruses in this group will prove to be readily transmissible mechanically to certain of their hosts.

SUMMARY

Several new hosts of the potato yellow-dwarf virus are reported.

The virus was shown to produce primary lesions upon *Nicotiana rustica* when leaves of a suitable age were inoculated by the carborundum method. This host has proved important in investigating properties of the virus.

Mechanical transmission of the potato yellow-dwarf virus from potato to potato or clover to clover proved difficult. The carborundum method was inefficient in effecting transmission on these hosts. The best method of mechanical inoculation on potato consisted of pin punctures into the tuber near an eye. On clover, transmission was secured by pin punctures into the crown.

The primary-lesion reaction was applied to the study of some properties of the virus. The virus in juice of *Nicotiana rustica* was destroyed at room temperature in from 2½ to 12 hours. It did not remain active in dried leaves. The virus could be recovered consistently from yellow-dwarf *N. rustica* juice diluted 10^{-3} with phosphate buffer or healthy juice, and in one experiment infections were obtained with a dilution of 10^{-5}. Its thermal inactivation point was found to be about 50° C. The virus passed through a Berkefeld W filter.

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STUDIES ON POLYPORUS ABIETINUS. II. THE UTILIZATION OF CELLULOSE AND LIGNIN BY THE FUNGUS[1]

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Cellulose and lignin, the two most important constituents of wood, comprise approximately 50 per cent and 30 per cent, respectively, of the dry weight of coniferous woods. Materials present in this magnitude, therefore, must play important rôles in the metabolism of wood-destroying fungi. Xylophilous fungi, consequently, have been classified into 2 groups, the brown-rot fungi and the white-rot fungi. This classification is based upon the supposition that certain fungi destroy only cellulose, leaving the brown lignin, and that others destroy only lignin, leaving the white cellulose. Falck and Haag (3) proposed the term "destruction fungi" for the brown-rot group, and "corrosion fungi" for the white-rot group. Campbell (2), however, demonstrated that wood decayed by single species is lower both in cellulose and in lignin than undecayed wood, and, consequently, that certain fungi are able to destroy both cellulose and lignin. Among the fungi regarded by Campbell as destroying both of these constituents simultaneously, is *Polyporus abietinus* (Dicks.) Fries. This fungus, however, had previously been classified by Thaysen and Bunker (6) as a brown-rot fungus and by Hubert (5) as a white-rot fungus. The purpose of the study here reported was to test the ability of *P. abietinus* to utilize cellulose or lignin, singly as a source of nutrition. If it is able to utilize both materials in culture it may be assumed that, when growing in wood, it could also destroy both materials. If, however, it is able to utilize only one or the other of the constituents then it could not be anticipated that both serve as food under natural conditions.

Methods

In testing the ability of *Polyporus abietinus* to utilize either cellulose or lignin as a source of nutrition, the fungus was grown on media containing either cellulose or lignin as the only organic nutritional material. For this purpose a mineral stock agar composed of the following ingredients was prepared:

Distilled water, 1,000.0 g.; $MgSO_4$, 0 5 g.; K_2HPO_4, 1.0 g.; KCl, 0.5 g.; $FeSO_4$, 0.1 g.; agar-agar, 20.0 g.

This stock agar, alone, did not support the growth of the fungus. To the stock medium, therefore, 2.5 per cent cellulose gel was added. This cellulose gel was prepared from filter paper by use of Schweitzer's reagent. A lignin medium was prepared by adding 2.5 per cent isolated lignin to the stock medium. This lignin was obtained from pine wood by use of the sulphuric

[1] A portion of a thesis submitted to the Graduate Faculty of Duke University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] The author herewith gratefully acknowledges his indebtedness to Dr. Frederick A. Wolf under whose guidance this work was conducted.

acid method of isolation. A portion of the cellulose medium and a portion of the lignin medium were further enriched by the addition of 1 per cent ammonium nitrate.

Poured plates of these media were inoculated with a vigorous culture of *Polyporus abietinus* and were incubated at 30° C. for the periods listed in table 1. At the end of this period the radial growth of the colonies was measured.

Bavendamm (1) advocated the use of another method for testing the ability of a fungus to utilize lignin. In this method the fungus is grown on a medium containing tannic acid. If a dark brown halo is formed, then it is assumed that the fungus is able to digest lignin. According to Bavendamm (1) lignin and tannic acid are closely related substances. The decomposition of tannic acid, shown by the formation of the brown decomposition products, is evidence that the similar substance, lignin, also can be destroyed. In the present study Bavendamm's method also was employed. For this purpose a tannic acid medium was made by adding 0.5 per cent tannic acid to the stock agar. Poured plates of this medium were inoculated with *Polyporus abietinus* and were incubated for the periods indicated in table 1.

RESULTS

The results of these cultural studies with *Polyporus abietinus* may be presented most concisely in the following table:

TABLE 1.—*Growth of Polyporus abietinus on cellulose, lignin, and tannic acid media*

Medium	No. of plates	Days growth	Average growth in millimeters	Character of growth
Stock agar and 2.5 per cent cellulose	11	9	29	fair
Stock agar and 2.5 per cent lignin	10	30	23	very sparse
Stock agar, 2.5 per cent cellulose, 1 per cent NH_4NO_3	10	9	28	fair
Stock agar, 2.5 per cent lignin, 1 per cent NH_4NO_3	11	30	30	sparse
Stock agar and 0.5 per cent tannic acid	12	9	12	good, dark brown halo

DISCUSSION

The growth of *Polyporus abietinus* on media containing either cellulose or lignin as a source of organic nutrition indicates that this fungus is able to utilize both of these components of wood. Thus the evidence obtained from cultural studies corroborates the evidence obtained by Campbell (2), for the simultaneous destruction of lignin and cellulose in wood by this fungus. It is evident, however, that cellulose *per se* is a better source of nutrition for the fungus than is lignin.

The exact nature of the lignin complex of wood is, at present, unknown.

It is fairly generally accepted, however, that it is composed largely of methoxyl, hydroxyl, acetyl, and phenolic groups. Energy would be released if these groups were acted upon by oxidizing enzymes, but no assimilable products would be furnished. According to Hawley and Wise (4), there also is evidence that pentosans are a part of the lignin complex, while others regard the pentosans as impurities resulting from the technique of isolation. The sulphuric acid employed in the isolation of the lignin for the present study should have hydrolyzed any pentosans that may have been loosely associated with the lignin. The products of this hydrolysis should have been removed in the subsequent washing of the residue. The growth of *Polyporus abietinus* on the medium containing the isolated lignin, consequently, indicates that there are materials intimately associated with the complex organic groups of the lignin that are released as sources of nutrition only after oxidizing enzymes have caused a partial breakdown of these complex organic groups. Such nutritional materials, however, are apparently present in very small amounts as evidenced by the poor growth of the fungus.

Obviously lignin isolated from wood is not identical with lignin in wood. Nevertheless, it is logical to assume that an organism capable of producing the enzymes necessary for the utilization of isolated lignin would also be able to utilize lignin in wood. *P. abietinus* grows better on the lignin medium enriched with ammonium nitrate than on the lignin medium not so enriched. It did not grow any better, however, on the cellulose medium enriched with ammonium nitrate than on the cellulose medium without the ammonium nitrate. This indicates that nitrogenous materials are of more importance in the utilization of lignin by the fungus than in the utilization of cellulose.

The formation of the brown halo on the tannic acid medium indicates that *Polyporus abietinus* forms the enzyme laccase. Gallic acid, a phenol resulting from the decomposition of the tannic acid, is oxidized by laccase with the resultant formation of dark brown pigment. The reason for assuming, as advocated by Bavendamm (1), that a wood-destroying fungus which produces a brown halo on tannic acid medium is a corrosion or white-rot fungus, and that one which does not produce the brown halo is a brown-rot fungus appears never to have been adequately explained. It seems probable that the enzyme laccase is produced by certain fungi, and that it catalyzes the oxidation of the phenolic groups of the lignin. The oxidation of these phenolic groups, therefore, constitutes a partial decomposition of the lignin complex. It should be emphasized, however, that the production of the brown halo on tannic acid medium need not be interpreted to show that the fungus utilizes lignin alone and leaves the cellulose intact. The present experiments with *P. abietinus* show that this fungus can utilize both lignin and cellulose, and therefore indicate that the Bavendamm test is not a specific means of distinguishing between the two groups of wood-destroying fungi.

SUMMARY

Polyporus abietinus utilizes either cellulose or isolated lignin as a source of nutrition. Cellulose is a better source of nutrition than is lignin.

Nitrogenous materials are of more importance in the growth of the fungus on lignin than in the growth of the fungus on cellulose.

The fungus forms a brown halo when grown on tannic acid medium, indicating the formation of the enzyme laccase. The laccase also probably catalyzes the oxidation of the phenolic groups in lignin, hence causing a partial decomposition of the lignin.

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THE INHERITANCE OF SCAB RESISTANCE IN CERTAIN CROSSES AND SELFED LINES OF POTATOES

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Common scab is widely distributed over the potato-producing areas of the world. The organism causing this disease, *Actinomyces scabies* (Thaxter) Güssow, is carried on seed potatoes and lives over from year to year in the soil. Control has been based on seed treatment. A number of seed treatments have been recommended that kill the organisms carried on the seed tubers, but no one has yet devised a method of treatment or a system of rotation that will completely eliminate scab infection arising from soil-borne organisms.

In recent years, therefore, potato breeders in the United States and elsewhere have been attempting to produce varieties resistant to the disease.

Experiments to determine whether any of the existing varieties were resistant have been made in many countries for many years. A few examples of the results will suffice to show that varieties have been known to differ in their reaction to the scab organism.

Among the early tests are those by Beckwith (1) in New York in 1887; Humphrey (5) in Massachusetts in 1890; Williams (12) in South Dakota in 1894 and 1895; and Shepperd and Ten Eyck (10) in North Dakota in 1902. In all of these experiments differences were found in the amount of scab on the tubers of the different varieties.

Stuart (11) found that although none of the 65 varieties tested were highly resistant to scab, some appeared to be less subject to the disease than others.

In tests of a large number of varieties by Schlumberger (8, 9) in Germany, a relatively high degree of resistance to scab was found in a small percentage of the varieties tested. Chief among these are Richter's Jubel, Arnica, Hindenburg, Oststarke, Robinia, Treff As, Rheingold and Ostragis.

The data at hand, covering a considerable range of varieties under widely different conditions, indicate that there are varying degrees of resistance to scab, although immunity has not yet been observed. Concerning this point, Berkner (2) says: "Absolute immunity does not appear to exist, but there are decided hereditary differences in the degrees of resistance and susceptibility."

The nature of resistance to scab has been studied by several investigators.

The fact that the most resistant American varieties possess a thick russet skin, has led some to believe that resistance is dependent upon this type of skin. Histological studies of several varieties by Lutman (7) led him to conclude that thickness of skin determines the resistance of the tubers to scab and that color is not important.

Stuart (11) pointed out the fallacy of the prevailing idea that the russet type of skin is the basis of freedom from scab and cites as an example the behavior of the Cambridge Russet, on the tubers of which scab was abundant during a period of 6 years.

In testing many seedlings, Darling, Leach, and Krantz (4) found a high degree of resistance in smooth and thin-skin seedlings, as well as in russet types. No correlation was found to exist between color of tuber and scab resistance.

Extensive studies by Longrée (6) of the skin structure of the tubers at different stages of growth showed no correlation between resistance to scab and time of periderm building, course of epiderm development, final thickness of skin, time of change of stomata to lenticels, or form, size, elevation, or depression of lenticels. Resistance was found to be associated with close packing of the "stuffing" cells of the lenticels, the degree of lenticel cambium corking, and limited ability to form wound periderm.

In the collection of seedling varieties studied by Darling, Leach and Krantz (4) the same characteristics were found associated with resistance as those reported by Longrée.

Resistance to scab is held by Wingerberg (13) to rest upon a purely physiological basis.

TESTS AT PRESQUE ISLE, MAINE

The research of the United States Department of Agriculture at Presque Isle, Maine, which was begun in 1930, has included tests of both American and foreign varieties, and progenies of a number of crossed and selfed lines. A preliminary report of this work by Clark *et al.* (3) shows that, among

the American varieties, Green Mountain, Irish Cobbler, Charles Downing, Warba, Katahdin, and Chippewa were highly susceptible to common scab and that Russet Rural, Russet Burbank, Mahr's Russet, Golden, American Giant, and an unnamed seedling, No. 44537, were intermediate in their reaction, and would be considered moderately resistant. None of the American varieties tested showed a high degree of resistance. On the other hand, the European varieties, Hindenburg, Richter's Jubel, Ackersegen, Arnica, and Hindenburg × Centifolia No. 9 were proved highly resistant. All of these introductions produced less than 1 per cent of the amount of scab found on the Green Mountain checks. None of them had enough lesions on the tubers to classify them as scabby, while the Green Mountain tubers in the check plots could not have been sold for first-class table stock.

Besides the named varieties, both American and foreign, a number of progenies have been tested during the last 4 years in an attempt to determine the genetic behavior of some of the resistant and susceptible sorts, and the possible combination of resistance to this disease with other characters of economic importance. The seedling variety used most extensively in the series of crosses presented in this paper is No. 44537. It is self-fertile, has a heavy russet skin, and is moderately resistant to scab. For these reasons, in part, it was used extensively in many of the early crosses, and, also, because its fertility made possible the production of selfed lines, crosses, and back crosses. It has, however, produced consistently low yields, and the lines obtained by selfing lack vigor; hence, other, more vigorous fertile sorts have replaced it in the recent breeding studies.

In the study of scab resistance in the various progenies the first scab data are secured from the seedlings grown from true seed. Although they are grown on nonlimed soil, a relatively severe epidemic usually prevails, so that it is possible to separate them into 2 classes, those with very little or no scab, indicating either escape or resistance, and those sufficiently scabby to clearly indicate susceptibility. The former are saved and planted in the scab test plots the following season. During the last 4 years the latter group of seedlings have been planted in soil known to have produced scabby tubers in previous years, which has received yearly applications of lime at the rate of about 1 ton per acre. Each seedling is grown in 5-hill plots, each hill alternating with a hill of Green Mountain, which is very susceptible to scab.

The method of taking the scab data has changed to some extent during the progress of the experiment, and further changes will be required before it is entirely satisfactory. In judging the reactions of the varieties and checks two criteria must be taken into consideration, the percentage of tuber surface covered with scab, and the type of pustule. Figure 1, which facilitates the reading and makes the estimates more reliable, shows tubers in 6 classes: 0, 10, 30, 50, 70, and 90 per cent of surface covered. This illustration was made by actual measurement. It is especially helpful in getting accurate readings on the Green Mountain checks, on which the reliability of the test depends. Figure 2 shows various types of scab lesions. Four types

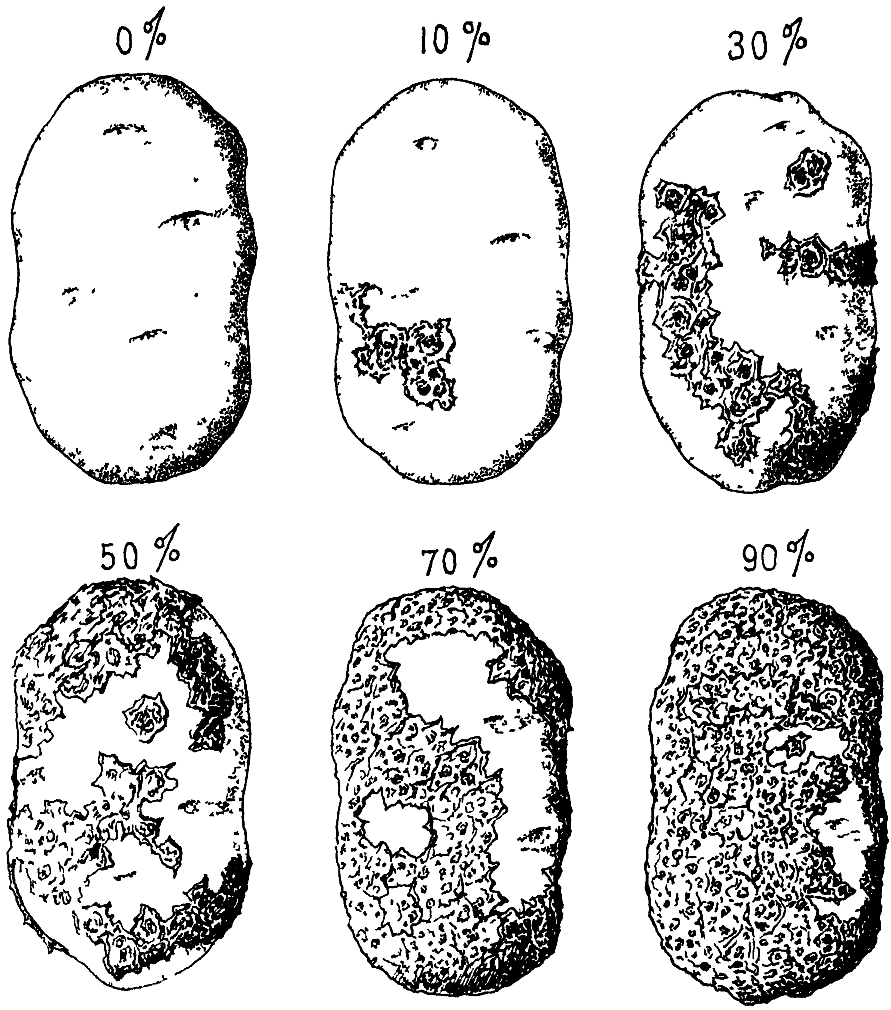

FIG 1 Tubers showing various percentages of surface covered with lesions of common scab

are represented, 3 pustule and one russeting type No 1 shows possible susceptibility in spite of the fact that only a few pustules are found on the surface, and would be considered susceptible if the Green Mountain check in the adjacent hills showed the same percentage of surface covered On the other hand, if the tubers of the seedling variety under test showed only a few scattered pustules of type 1, and the Green Mountain check showed a very much higher percentage, the seedling variety would be considered more resistant than the Green Mountain. Types 2 and 3 without doubt indicate resistance. Type 4 is the most difficult to evaluate. While it is quite certain that scab will produce this type, other factors of environment may cause a similar condition, and there may be confusion with natural russeting conditioned by genetic factors. Because so little is known concerning this type, it has not been taken into consideration in the data here presented.

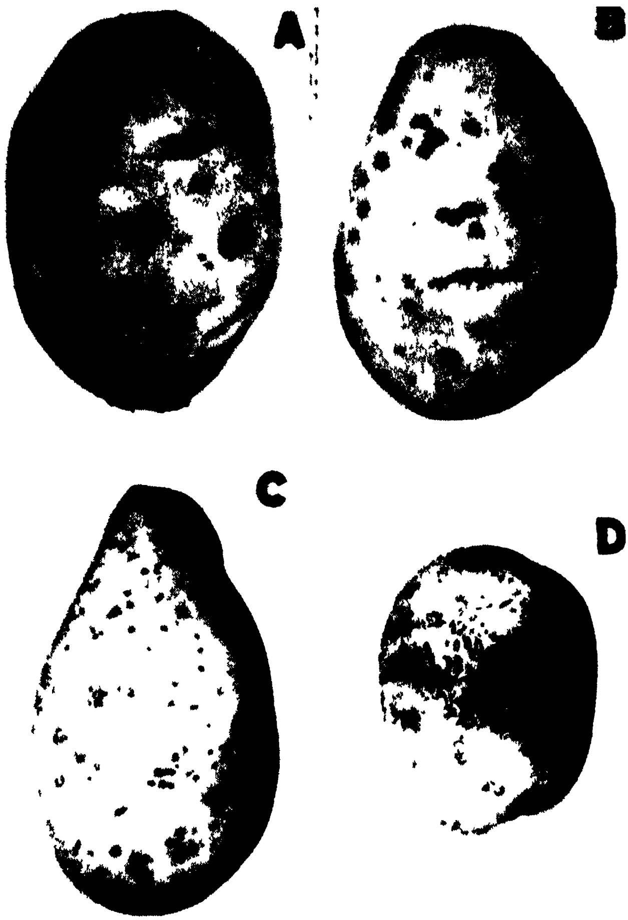

FIG. 2. Types of common scab found on potato varieties showing various degrees of resistance to the disease. A. Type 1. B. Type 2. C. Type 3. D. Type 4.

RESULTS

The progeny of a cross between 2 susceptible parents, Columbia Russet and Katahdin, were completely susceptible. The tubers of the 392 seedlings of this cross showed a high percentage of the surface covered with No. 1 pustules. In contrast to this, all of the 827 seedlings of the cross between 2 highly resistant varieties (Hindenburg × Ostragis) were as resistant as either of the parents. All of the other crosses segregated for resistance and susceptibility (Table 1). The progenies of all the crosses with at least one russet-skin parent segregated for russet and non-russet types of skin. The highly susceptible seedlings were discarded without further test. The seedlings showing little or no scab, however, were given a more severe test the following year. They were planted in soil that was known to have produced scabby tubers in previous years and to which

TABLE 1.—*Reaction to pustule types of common scab of the first-year-seedling progenies at Presque Isle, Maine*

Pedigree No.	Parents	Reaction of parents	Seedlings	Susceptible; discarded		Little or no scab; saved	
				Russet	Non-russet	Russet	Non-russet
			Number	*Number*	*Number*	*Number*	*Number*
× 146	Columbia Russet × Katahdin	Suscept. × suscept.	392		392		
× 277	Columbia Russet × S 44537	Suscept. × mod. resist.	420	61	224	111	24
× 295	Russet Rural × S 44537	Mod. resist. × mod. resist.	350	34	201	79	36
× 528	Richter's Jubel × S 44537	Resist. × mod. resist.	621	4	211	216	190
× 574	Arnica × S 44537	do	70	5	37	17	11
× 920	S 44537 × S 46422	Mod. resist. × suscept.	271	51	133	67	20
× 1037	S 45208 × S 44537	Suscept. × mod. resist.	354	68	178	58	50
× 161	Richter's Jubel × Earlaine	Resist. × suscept.	61		10		51
× 570 × 1002	Hindenburg × Ostragis	Resist. × resist.	827		0		827
× 579	Rural New Yorker × S 44537	Suscept. × mod. resist.	457	27	298	92	40
× 601	Arnica × Ostragis	Resist. × resist.	20		2		18
× 627	Hindenburg × Katahdin	Resist. × suscept.	900		428		472

TABLE 2.—*Reactions to pustule types of common scab of seedlings that showed little or no scab in 1935; grown from tubers in 1936 in limed soil with Green Mountain checks in alternate hills*

Pedigree No.	Parents	Skin character	Classes of infection						Total No. seedlings	χ^2 test for each group compared with its checks
			0	1–20	21–40	41–60	61–80	81–100		P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
× 277	Columbia Russet × S 44537	Russet		64	33	6	3		106	Less than 1 per cent
	Green Mountain check	White				19	26	61	106	
	Columbia Russet × S 44537	do		5	2	6	7	4	24	Approximately 25 per cent
	Green Mountain check	do				6	6	12	24	
× 295	Russet Rural × S 44537	Russet		59	18				77	Less than 1 per cent
	Green Mountain check	White				24	47	6	77	
	Russet Rural × S 44537	do			9	20	7		36	Approximately 7 per cent
	Green Mountain check	do				17	18	1	36	
× 528	Richter's Jubel × S 44537	Russet		165	40	10	1		216	Less than 1 per cent
	Green Mountain check	White				29	94	93	216	
	Richter's Jubel × S 44537	do		35	90	62	3		190	Less than 1 per cent
	Green Mountain check	do				30	85	75	190	
× 574	Arnica × S 44537	Russet		10	5	2			17	Less than 1 per cent
	Green Mountain check	White				5	9	3	17	
	Arnica × S 44537	do			7	3	1		11	Approximately 8.0 per cent
	Green Mountain check	do				7	4		11	
× 920	S 44537 × S 46422	Russet		50	13	3			66	Less than 1 per cent
	Green Mountain check	White				5	18	43	66	
	S 44537 × S 46422	do			1	16	2	1	20	Less than 1 per cent
	Green Mountain check	do					9	11	20	
× 1037	S 45208 × S 44537	Russet			36	20	1		57	Less than 1 per cent
	Green Mountain check	White				19	19	19	57	
	S 45208 × S 44537	do			2	27	16	6	51	Approximately 6 per cent
	Green Mountain check	do				11	21	19	51	
Inb. 1241	Katahdin selfed	do			3	28	10	9	50	Approximately 1 per cent
	Green Mountain check	do				12	8	30	50	

lime had been applied each year at the rate of about 1 ton per acre. Susceptible Green Mountain was planted in alternate hills. The russet and non-russet seedlings of each cross are grouped separately in tables 2 and 3, each group being followed by its Green Mountain check. Each group was compared with its checks by the χ^2 test for goodness of fit on the basis that, so far as reaction to scab is concerned, the seedlings and their checks could both be samples of a population the parameter of which is the mean of the 2 samples. The P values are given in the tables. In every progeny the russeted segregates were more resistant than the Green Mountain variety, the differences being highly significant. The white segregates showed on the average less resistance than their russeted sibs. In crosses 277, 295, 574, and 1037 significant differences between the white segregates and their Green Mountain checks could not be demonstrated. In all the other crosses the differences between them were highly significant.

The reaction of 3 selfed lines, seedling No. 44537 selfed, Katahdin selfed, and Green Mountain naturally pollinated, is interesting. The latter is considered a selfed line, since the seed was obtained in a field of Green Mountains with no fertile varieties in close proximity. Seedling 44537 is moderately resistant to scab. It will be noted that it is one of the parents of most of the crosses reported in this paper. A selfed line of this variety segreated for russet and white skin and for resistance and susceptibility to scab. Both the russet-skin segregates and their white-skin sibs differed significantly from Green Mountain (Table 3). Katahdin is as susceptible to scab under the conditions of these tests as Green Mountain. It breeds true for white skin, yet the probabilities are less than 1 in 100 that the progeny of Katahdin, selfed, and the Green Mountain variety react alike to the pustule types of scab (Table 3). On the other hand, the progeny of Green Mountain selfed and the same number of lots of the Green Mountain variety could be drawn 90 per cent of the time from a population of which the mean of the two is the parameter.

It is evident that in these progenies russeting is associated to a high degree with resistance to the pustule types of scab. The complete data for several segregating progenies with the P values found in comparing the russet segregates with their white-skin sibs are given in table 4. In 6 crosses the probabilities are less than 1 in 100 that the russet and white-skin types belong in the same population, as far as scab resistance is concerned. That there is not a complete correlation between russeting and scab resistance is shown by the fact that in each of the crosses a number of russeted segregates were found completely susceptible to the disease. One cross, Hindenburg × Ostragis, segregated for red and white color of tubers. This whole progeny was highly resistant to scab (Table 3). It can be seen without applying the goodness-of-fit test that these two groups are almost exactly alike in their reaction to scab, and that scab resistance, therefore, is not correlated with color of tubers in this cross. If the progeny of Katahdin, selfed (Table 2), is compared with the white segregates of the

TABLE 3.—*Reactions to pustule types of common scab of seedlings that showed little or no scab in 1936; grown from tubers in 1937 in limed soil with Green Mountain checks in alternate hills*

Pedigree No.	Parentage	Skin character	Classes of infection							χ^2 test for each group compared with its checks
			0	1–20	21–40	41–60	61–80	81–100	Total	P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
× 161	Richter's Jubel × Earlaine	White	4	23	1	1			29	Less than 1 per cent
	Green Mountain check	do			6	23			29	
× 579	Rural N. Y. × S44537	Russet	66	9	2	1			78	do
	Green Mountain check	White		1	11	38	27	1	78	
	Rural N. Y. × S44537	do	1	8	13	7	1		30	Approximately 2 per cent
	Green Mountain check	do			5	15	10		30	
× 601	Arnica × Ostragis	Not russet	12	4					16	Less than 1 per cent
	Green Mountain check	do		1	3	8	4		16	
× 627	Hindenburg × Kat.	White	138	152	11				301	do
	Green Mountain check	do		2	80	160	44	15	301	
× 627S[a]	Hindenburg × Kat.	do	114	10					124	do
	Green Mountain check	do		70	44	10			124	
× 570[a] and	Hindenburg × Ostragis	Red	333	4					337	do
	Green Mountain check	White		143	129	47	14	4	337	
× 1002[a]	Hindenburg × Ostragis	do	343	4					347	do
	Green Mountain check	do		161	116	43	15	11	347	
Inb. 1364[a]	S44537 selfed	Russet	16	10	1				27	do
	Green Mountain check	White		5	15	7			27	
	S44537 selfed	do	8	23	7	1			39	Approximately 2 per cent
	Green Mountain check	do		8	12	17	2		39	
Inb. 1241	Katahdin selfed	do		40	45	18	1		104	Less than 1 per cent
	Green Mountain check	do		1	8	66	29		104	
	Green Mountain nat. pol.	do	1	30	15	2			48	Approximately 90 per cent
	Green Mountain check	do		27	15	6			48	

[a] These selections were planted in single hills alternating with Green Mountain; all other seedlings were planted in rows in which 5 hills of the seedlings alternated with 5 hills of Green Mountain.

cross Richter's Jubel × Seedling No. 44537, the probabilities are less than 1 in 100 that they are samples from the same population. There are evidently more factors or more potent factors for resistance involved in the Richter's Jubel × Seedling No. 44537 cross than in selfed Katahdin.

As was to be expected, there was a great difference between severity of scab epidemics in different years. This is shown by the 1936 and 1937 data for 42 white skin seedlings of the cross Richter's Jubel × Seedling No. 44537 and the corresponding Green Mountain checks (Table 5). If the Green Mountain checks for the year 1936 are compared with those for 1937 by the chi-square test, the probabilities are much less than 1 in 100 that they are alike. The various seedling varieties, however, showed about the same degrees of resistance in relation to Green Mountain in 1937 that they did in 1936, the probabilities in each year being less than 1 in 100 that the seedling varieties and the Green Mountain checks were alike in their reactions to scab.

None of the resistant foreign introductions or seedlings developed in the early years of these investigations have approached the commonly grown commercial varieties in vigor and yield, but some of the recent productions resulting from hybridization and selection are more promising. One of these is shown in figure 3. This is a white-skin variety, a selection

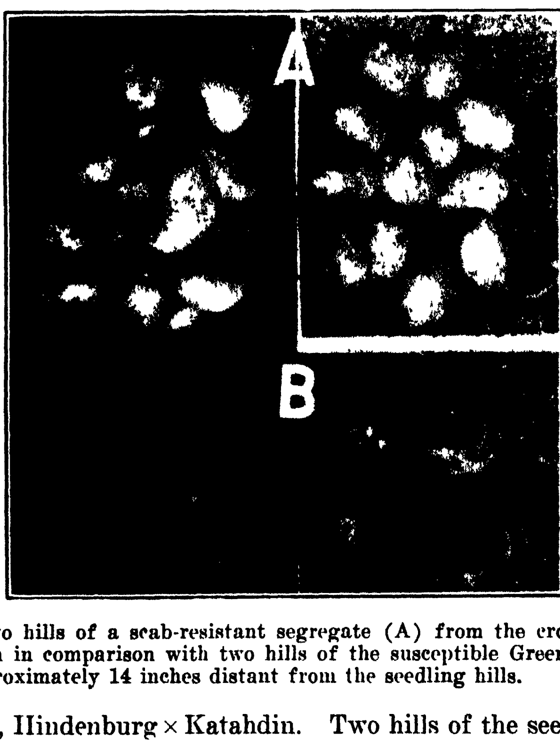

FIG. 3. Two hills of a scab-resistant segregate (A) from the cross, Hindenburg × Katahdin, shown in comparison with two hills of the susceptible Green Mountain check (B), grown approximately 14 inches distant from the seedling hills.

from the cross, Hindenburg × Katahdin. Two hills of the seedling, A in the picture, are shown in contrast to two hills of the Green Mountain variety B grown in alternate hills approximately 14 inches apart in the row. This

TABLE 4.—*Comparison between russet-skin segregates and their white-skin sibs in their reaction to pustule types of common scab*

Pedigree No.	Parents	Skin character	Classes of infection								χ^2 test of russet-skin resistance compared with white
			0	1–20	21–40	41–60	61–80	81–100	Susc. discarded as seedlings	Total	P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*			
× 277	Columbia Russet × S44537	Russet		64	33	6	3		61	167	Less than 1 per cent
	do	White		5	2	6	7	4	224	248	
× 295	Russet Rural × S44537	Russet		59	18				34	111	do
	do	White			9	20	7		201	237	
× 579	Rural New Yorker × S44537	Russet	66	9	2	1			27	105	do
	do	White	1	8	13	7	1		298	328	
× 528	Richter's Jubel × S44537	Russet		165	40	10	1		4	220	do
	do	White		35	90	62	3		211	401	
× 574	Arnica × S44537	Russet		10	5	2			5	22	Approximately 5 per cent
	do	White			7	3	1		37	48	
× 1037	S45208 × S44537	Russet			36	20	1		68	125	Less than 1 per cent
	do	White			2	27	16	6	178	229	
× 920	S44537 × S46422	Russet		50	13	3			51	117	Less than 1 per cent
	do	White			1	16	2	1	133	153	
Inb. 1364	S44537 selfed	Russet	16	10	1					27	Exceeds 30 per cent
	do	White	8	23	7	1				39	

seedling is all that can be desired from the standpoint of resistance under the conditions of this test, but it sets too many tubers for ordinary growing conditions and the tuber shape is not the most desirable.

TABLE 5.—*Resistant white-skin seedlings selected from the 1936 test and tested again in 1937*

Pedigree No.	Parents	Year	Classes of infection						Total no. seedlings
			0	1–20	21–40	41–60	61–80	81–100	
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
×528	Richter's Jubel ×344537	1936			30	12			
	Green Mountain check	1936					15	27	42
×528	Richter's Jubel ×S44537	1937	18	21	3				42
	Green Mountain check	1937		2	5	25	10		42

DISCUSSION AND SUMMARY

Although more research will be necessary before a genetic interpretation of the inheritance of common scab resistance in potato can be made, a number of facts of importance to the potato breeder have been demonstrated in the present investigation. Green Mountain apparently breeds true for susceptibility to scab. Katahdin is susceptible but carries at least one factor for scab resistance in a heterozygous condition. Hindenburg and Ostragis are probably homozygous for resistance. Seedling No. 44537 and Richter's Jubel are heterozygous for scab resistance. There is a genetic linkage between russeting and resistance. In one cross the resistant factors were independent of red and white tuber colors. Russet, red, and white-skin types have been produced that are highly resistant to scab.

A number of white-skin seedling potato varieties have been produced by hybridization and selection that are highly resistant to scab under conditions of the tests at Presque Isle, Maine. Some of these approach commercial varieties in vigor and yield (Fig. 3), but are inferior in other characters. There is every reason to believe, however, that by using these resistant sorts as parents, and by selfing, sib-mating, and back-crossing, new varieties superior to the commonly grown commercial sorts can be produced.

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FURTHER STUDIES ON HOST RELATIONSHIPS OF PEACH MOSAIC IN SOUTHERN CALIFORNIA[1,2]

L. C. COCHRAN AND LEE M. HUTCHINS

(Accepted for publication Aug. 23, 1938)

Mosaics of almond, apricot, plum, and prune, occurring naturally in southern California, have been reported by the writers to produce peach-mosaic-like symptoms in peach after graft inoculations.[3] In the paper here presented, further observations and experiments on host relationships of peach mosaic are reported, emphasis being given to results of inoculations of peach mosaic into almond, apricot, plum, and prune.

Healthy apricot nursery trees were inoculated by budding from typically mosaic-affected commercial J. H. Hale peach trees, in September, 1937. In all cases, the inserted peach buds (inoculum) made union, and growth from these buds showed severe peach-mosaic symptoms in the spring of 1938, but no mosaic symptoms had been manifested in apricot growth of these trees up to the time this paper was prepared (June, 1938). In the spring of 1938, healthy J. H. Hale test-scions were grafted on limbs of these trees a few feet from the point of inoculation. The test-scions developed symptoms of peach mosaic shortly after growth started, showing that under the conditions of the experiment peach-mosaic virus was able to pass through the apricot trees, after artificial inoculation, without producing symptoms in this species. In healthy plum and prune nursery trees that were inoculated as were the apricot trees, growth from the mosaic-infected peach buds (inoculum) showed typical symptoms of peach mosaic throughout the spring of 1938, whereas the plum or prune growth of these trees showed no mosaic symptoms. Healthy J. H. Hale test-scions grafted on these trees in the spring of 1938, a few feet from the point of inoculation, behaved variously. Up to June 9, about 75 per cent of these scions had developed typical peach mosaic and about 25 per cent were still normal. It is possible that the virus passed more slowly through plum and prune than it did through apricot, and that all of these peach test-scions on plum or prune eventually may show peach mosaic symp-

[1] Paper No. 390, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Cooperative investigations between the University of California Citrus Experiment Station and the Bureau of Plant Industry of the U. S. Department of Agriculture.

[3] Cochran, L. C., and Lee M. Hutchins. Peach-mosaic host-relationship studies in southern California. (Abstract.) Phytopath 27: 954. 1937.

toms. In check experiments, where healthy almond, apricot, plum, and prune nursery trees were not inoculated with mosaic-infected peach buds but were indexed with healthy J. H. Hale test-scions, growth from all test-scions was normal throughout the spring of 1938, showing that no latent virus capable of producing peach-mosaic-like symptoms in peach was present in the trees before the experiments were set up.

Nonpareil almond nursery trees, on which peach-mosaic-affected J. H. Hale scions from diseased orchard trees were grafted by H. S. Reed, in 1935, have developed no symptoms of peach mosaic in the almond growth, even in cases where growth from the peach scion (inoculum) has attained 4 to 5 feet in length and has shown severe peach mosaic symptoms during the period 1935–1938. Healthy J. H. Hale peach test-scions, grafted on the almond wood of these trees in 1937 or 1938, have developed characteristic symptoms of peach mosaic, showing that the virus may be present in the almond for at least 3 years without producing mosaic symptoms.

When buds or scions from orchard apricot trees showing typical apricot mosaic as it occurs naturally were grafted on J. H. Hale peach nursery trees, symptoms identical with peach mosaic in J. H. Hale were produced. Inoculation back to apricot nursery trees, through grafts from the J. H. Hale trees thus infested, produced typical apricot mosaic in the apricot trees. Similar experiments with naturally occurring almond, plum, and prune mosaics are in progress but are not sufficiently mature for records.

Naturally occurring mosaics of almond, apricot, and prune were transmitted from diseased to normal trees of the respective species, the incubation period being similar to that for peach mosaic in peach. Naturally occurring almond mosaic in orchard trees, when inoculated into healthy apricot nursery trees, produced in the latter species symptoms typical of apricot mosaic.

Surveys of commercial plum and prune orchards of large trees, contiguous to commercial peach orchards in which 75 per cent of the peach trees showed peach mosaic, revealed no mosaic symptoms in the plum or prune trees. In 5 such instances, rows of plum and prune trees were indexed by grafting on each tree a healthy J. H. Hale peach scion, or by grafting a bud from each orchard tree into a healthy J. H. Hale peach nursery tree. The test-scions and the nursery trees grew normally in all cases and showed no mosaic symptoms, indicating that peach mosaic had not spread naturally into the plum or prune trees in these instances. Indexing of the plum part of trees resulting from topworking mosaic-affected peach trees with plum scions showed the peach-mosaic virus present in the plum top in all cases. Surveys of old French Prune orchards contiguous to apricot and almond orchards heavily infected with naturally occurring mosaics of these species revealed that only 1 to 2 per cent of the prune trees were mosaic-affected. Indexing of these orchards by budding from each tree to a healthy J. H. Hale nursery tree showed that infection was present in the prune trees only where the latter showed symptoms.

These data, shown graphically in figure 1, establish the fact that, while

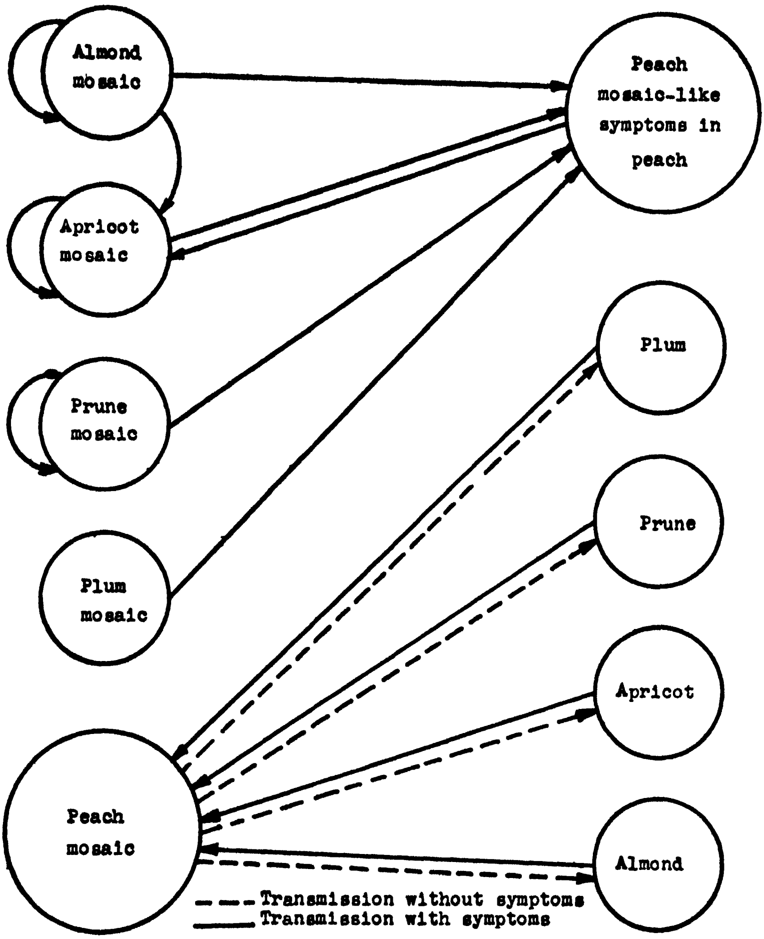

FIG. 1. Graphic outline showing results of cross-inoculation studies with naturally occurring stone fruit mosaics in southern California, as far as completed by June, 1938.

certain naturally occurring mosaics of almond, apricot, and plum are capable of producing peach-mosaic-like symptoms in peach by means of graft inoculations, a reciprocal relationship may not be true. When naturally occurring peach mosaic in peach is inoculated into almond, apricot, and plum, these species may act as symptomless carriers of the peach-mosaic virus.

While this evidence would seem to indicate that more than one mosaic-producing virus of *Prunus* species may be present in southern California, it is difficult to explain why all of these mosaics should produce symptoms in peach indistinguishable from peach mosaic in peach. The difficulty is further increased by survey reports indicating that the natural distribution of these mosaics in southern California is correlated in a general way with the presence of mosaic-affected peach trees. The possibility of virus strains must be considered, and the authors feel that further experimentation will be necessary before definite conclusions can be drawn.

A NEW ENTOMOGENOUS FUNGUS ON THE CORN EARWORM, HELIOTHIS OBSOLETA

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(Accepted for publication July 22, 1938)

While cooperating in the study of the fungus, *Sorosporella uvella* (Krass.) Gd., parasitic on the corn earworm, Mr. F. F. Dicke, Bureau of Entomology and Plant Quarantine, uncovered fungus-infected pupae from hibernation cages at the Arlington Experimental Farm of the Bureau of Plant Industry at Arlington, Virginia. These specimens appeared to be very different from those attacked by *Sorosporella uvella,* and a microscopic examination showed this to be true.

The first collection was made in May, 1936, and subsequent collections from the same locality in May and November of 1937, and in March, 1938.

Specimens of this fungus were also collected by Mr. Dicke at Moorestown, New Jersey,[1] and McLean, Virginia, in March, 1938. Material was also received from Mr. A. F. Satterthwait of the Bureau of Entomology and Plant Quarantine, in 1937. This collection was made at 2 canneries at Hoopeston, Illinois, where the worms had been observed to be dying.

MACROSCOPIC APPEARANCE OF THE DISEASED INSECTS

There was no external evidence of the fungus in most of the spring collections of diseased pupae. The color was normal and the bodies remained unshrunken. In a few instances, however, the fungus could be seen emerging from the sutures and forming a cobwebby mass on the exterior of the body. Specimens of diseased pupae collected in the fall were frequently found encased in a smooth, white, mycelial sheath. The presence of a similar development has been observed in other species of entomogenous fungi (Fig. 1). Fron,[2] in describing *Spicaria verticillioides,* which

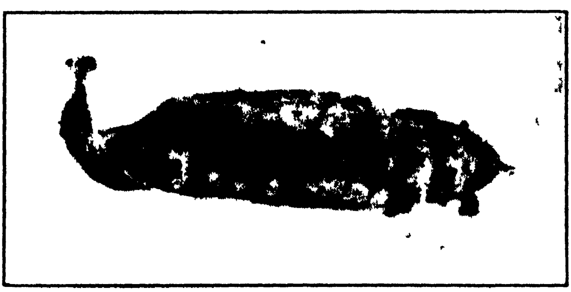

FIG. 1. Pupa of corn earworm covered with a mycelial sheath of the invading fungus. ×2.

[1] The larvae from which the pupae developed at Moorestown were collected in Fairfax County, Virginia, and placed in hibernation cages at Moorestown, New Jersey, in September, 1937. There is a possibility that the fungus was present in the larval stage, and transported to Moorestown.

[2] Fron, G. Note sur quelques Mucédinées observées sur *Cochylis ambiguella.* Bull. Soc. Myc. France 27: 482–487. 1911.

he later transferred to *Spicaria farinosa verticillioides*,[3] stated that the chrysalis presented the appearance of being covered with a white mold.

GENERAL DISCUSSION

No fungus fructification was observed on any freshly collected diseased pupae. The bodies of the pupae, however, contained a mass of tightly packed mycelium that had caused the complete obliteration of nearly all the organs. The alimentary canal alone was uninvaded.

In the younger material there was observed, in addition to the well-developed mycelium, blastocysts, similar in morphology to those described by Speare[4] in his studies of *Sorosporella uvella.*

Neither the mycelium filling the bodies of the pupae nor that from the sheath surrounding them gave a clue to the identity of the fungus parasite. It was, therefore, necessary to culture the material in order to ascertain whether the mycelium in the bodies of the pupae and that producing the mycelial sheath represented identical or different fungi and to determine the taxonomic position of the fungus or fungi involved.

CULTURAL CHARACTERS

Cultures of the fungus were made from the mycelium filling the body cavity and from the mycelial sheath. The organism grew readily on various media, but the type of growth differed consistently according to the kind of media used. On Molisch, potato, and corn-meal agar, in both Petri dishes and test tubes, the growth was white, sometimes zonate and slender clavae were produced (Fig. 2, A and B). The latter when quite mature adhered to the sides of the test tube or Petri dish, and showed a slight yellowish tinge. The clavae produced in the center of the cultures remained white and showed no coloration whatever. In cultures grown on wort, the clavae were broadly fan-shape and flattened (Fig. 2, C).

The method of fructification was distinctive, according to the culture medium on which the fungus was grown.

On corn meal, potato agar, and Molisch the fructification was developed uniformly over the entire surface of the clavae, while, on wort, the fungus fruited principally at the base of the clavae and only sparingly along the length of the clavae. Fructifications developed from the mycelial sheath also were produced in this manner.

A very luxuriant development of mycelium was produced from pupae encased in the mycelial sheath and kept at a low temperature in a tin container lined with moist filter paper. At first this growth was snow-white and byssoid, but gradually produced irregular flabelliform clavae. As in the wort cultures, the conidiophores were developed more frequently on the smaller clavae, and sparingly over the surface of the growth (Fig. 3, A

[3] Fron, G. Sur une Mucédinée de la Cochylis. Bull. Soc. Myc. France 28: 151–154. 1912.

[4] Speare, A. T. Further studies of *Sorosporella uvella*, a fungus parasite of noctuid larvae. Jour. Agr. Res. [U. S.] 18: 399–440. 1920.

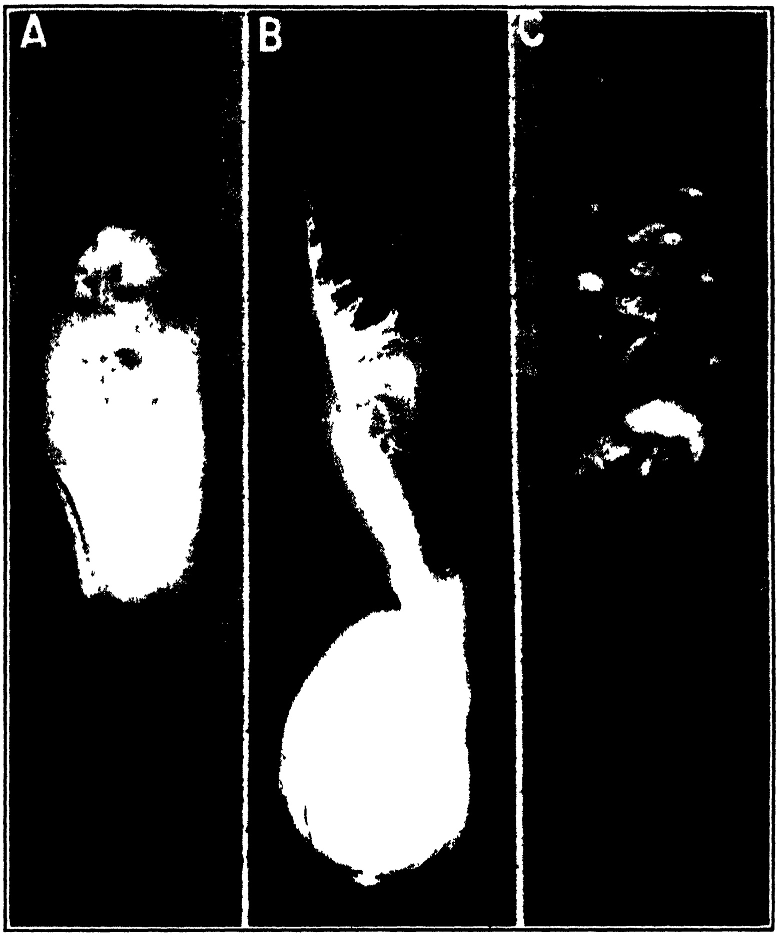

FIG. 2. Cultures of the corn earworm fungus. × 1¼. A. On Molisch showing zonate growth. B. On Molisch showing development of slender clavae. C. On wort showing fan shape clavae.

and B). The fungus on the pupae in the tin boxes grew luxuriantly and continuously for over 5 months, with only the addition of a few drops of water from time to time to keep the filter paper slightly damp. The fungus, developed from the pupae in the boxes, remained snow-white for over 6 weeks, when it began to show a slight lemon color.

IDENTITY OF THE FUNGUS

Very few species of fungi have been reported as occurring on the corn earworm, either as parasites or as secondary agents. A search of the literature failed to locate a species identical with the fungus occurring on the pupae of *Heliothis obsoleta*. The type of fructification is typical of the genus *Spicaria,* but, as in the case of certain other entomogenous fungi, normally classed as Mucedinaceae, the formation of synnemata places the fungus among the Stilbaceae. This fungus has been cultured continuously

FIG. 3. Culture developed from the mycelial sheath of the invading fungus; conidial formation on small clavae A and B. Photographed by M. L. F. Foubert.

for over 2 years in the hope of developing an ascogenous stage, but only the conidial form has been produced. In 1934 a specimen of *H. obsoleta* was received from Richmond, Virginia, which bore a prolonged sterile club suggestive of the genus *Cordyceps;* but there was no evidence of perithecia or remains of a conidial stage with which to make comparison.

Several species of *Spicaria,* some under the generic name of *Isaria* have been described on insects. Of these species *Spicaria farinosa verticillioides* approaches the form on *Heliothis* most closely, but it is believed that the fungus considered here is sufficiently distinct to be considered a new species.

DESCRIPTION OF THE FUNGUS

The color of the mycelium present in the bodies of the pupae was white to cream and consisted of segments of one to several cells. The latter presented considerable variation in length, but were fairly uniform in diameter. The blastocysts were observed intermingled with the mycelium, intact or in the process of germination.

As previously mentioned the development of synnemata seemed to be influenced by the kind of media on which the fungus was grown. On wort the clavae were flabelliform, broader, and more effuse than when grown on Molisch, potato, or corn-meal agar, and measured from 1 to 1½ cm. in height and were fully as broad in diameter. In addition the fructification was sparingly developed and more generally basal than over the entire surface of the synnemata. In the cultures grown on Molisch, potato, and corn-meal agar, in both test tubes and Petri dishes, the fructification was more typically stilbaceous, and the conidiophores developed uniformly over the entire surface of the synnemata. On these media, single or branched synnemata were developed on the sides of the tubes or Petri dishes and ranged from 1 to 2 cm. in height. The surface growth was pure white and more or less zonate (Fig. 2, A).

In the study here reported, the fungus presented considerable variation. An effort has been made to cover these differences in the specific description.[5]

On potato and corn-meal media the method of fructification was very typical of the genus *Spicaria*. The conidiophores bore 3 to 8 whorls of phialides and long chains of spores. As the cultures matured the number of whorls became fewer and more irregular and the verticillate arrangement less marked. The generic name of the host *Heliothis* has been used for the specific name of the fungus, which is accordingly designated **Spicaria heliothis**, n. sp.

Spicaria heliothis

Mycelium white, septate; synnemata solitary or gregarious, 1–1.5 cm. high; conidiophores simple or branched, erect, bearing 3–8 whorls of prophialides 3–5 verticillate or irregularly grouped, oblong, 7–8 μ; phialides globose with short sterigma; conidia variable in shape, ovate-elliptical, in long chains, 3–3.5 × 5–7 μ.

Mycelia albo, septato; synnematibus solitariis vel aggregatis, 1–1.5 cm altis; conidiophoris simplicibus vel ramosis erectis, 3–8 verticilla prophialidium gerentibus; prophialidibus 3–5 verticillatis vel conglomeratis, oblongis 7–8 μ; phialidibus globosis sterigmatibus breviusculis; conidiis variabilibus, ovato-ellipsoideis, in catenulas longas, 3–3.5 × 5–7 μ.

In culturis ex Heliothe obsoleta.

Type deposited in the Mycological Collections of the Bureau of Plant Industry.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

[5] It may avoid confusion in any future study of the genus *Spicaria* on *Heliothis* or other insect hosts, to call attention to the fact that during the course of the present investigation several species of this genus were encountered on the corn earworm. One very striking form, observed only once, was a robust, loosely branched type, having large phialides and spores. Another species, encountered only once, was a small and delicate form, pale vinaceous fawn in color. In addition to these 2 forms, an isolation was made from one of the Hoopeston, Illinois, collections, typical of *Spicaria farinosa verticilloides*. This species was not isolated from insects with the fungus discussed in this paper, but appeared to be the only parasite of the material from which it was cultured.

THE 1938 CROWN RUST EPIDEMIC OF OATS IN ARKANSAS IN RELATION TO HYBRIDS OF BOND AND VICTORIA

H. R. ROSEN AND L. M. WEETMAN

(Accepted for publication July 16, 1938)

In the December 1937 meeting of The American Phytopathological Society, Moore, Downie, and Murphy[1] reported a new race of crown rust, *Puccinia coronata avenae* F. and L., that attacks Bond oats (to be designated race 45, according to communications between H. C. Murphy and the senior writer). This race appeared on Bond and its hybrids at the Minnesota Agricultural Experiment Station and, according to the authors, was brought in, either on 2 collections made in Texas in the spring of 1937, or on material representing race 1, sent by H. C. Murphy to the Minnesota Station.

What appears to be the same race was found and identified by the present writers in Arkansas in 1937. In the process of identifying the races of crown rust present in Arkansas, from collections gathered by the writers in May and June, 1937, in all of the important oat-growing counties of the State, 2 single-pustule inoculations were made in October, 1937, on Markton oats from Collection No. 129, gathered on Bond at the Arkansas Experiment Station farm, Fayetteville. On November 17, when the second series of single-pustule inoculations on Markton were fully developed, the duplicate cultures were inoculated on the series of differential varieties that Murphy[2] had selected for the identification of races of crown rust. Both of these cultures produced within 10 to 14 days, a type-4 reaction on all the differentials, including Bond, except Glabrota, which yielded an I reaction, and Victoria, which showed a slightly greater susceptibility to this collection than it does to most collections of race 1. To make certain of these reactions, the duplicate single-pustule cultures were inoculated again on all the differentials and the same results obtained. It was apparent that a race of crown rust had been found that attacks Bond severely, and is mainly distinguished from race 1 by its reaction on this variety.

Out of 153 collections, race 45 subsequently was isolated in duplicate from 25 of them, while in 41 additional collections it appeared either in one or both of the duplicate isolations in amounts on Bond suggesting greenhouse contamination. The counties in which race 45 appeared in duplicate from single collections and apparently free from contaminations, were Carroll, Clay, Crawford, Cross, Franklin, Lee, Madison, Marion, Poinsett, and Washington. Most of these are in northern Arkansas.

This new race, and race 1, was inoculated on some 3,700 Victoria hybrid

[1] Moore, M. B., A. R. Downie and H. C. Murphy. A new race of crown rust that attacks Bond oats. (Abstract.) Phytopath. 28: 16–17. 1938.

[2] Murphy, H. C. Physiologic specialization in *Puccinia coronata avenae*. U. S. Dept. Agr. Tech. Bull. 433. 1935.

plants grown in the greenhouse, while a similar number of Bond hybrids were inoculated with race 1 only. Within several weeks contaminations with the new race became increasingly common on all the Bond hybrids, despite serious efforts to prevent them. Lack of greenhouse space made it impossible to segregate the Bond hybrids from the others and to keep the inoculated plants and incubating chambers in rooms separated from healthy plants. There appeared to be good evidence for the conclusion that under greenhouse conditions race 45 is far more aggressive than race 1.

Contamination of these Bond hybrids with race 45 was most discouraging from the standpoint of breeding for crown-rust resistance. Although overhead watering was carefully avoided and the leaves kept free from water, many of the plants suffered a loss of 50 per cent or more of their leaf areas through rust infections before and during heading. The ease with which this race spread in the greenhouse seemed ominous for Bond and its hybrids, under field conditions.

Fortunately for the breeding program, the 1938 season offered almost ideal field conditions at Fayetteville, Arkansas, for natural dissemination and infection, so that Bond and its hybrids, as well as all other varieties, were subjected to a severe epidemic of crown rust. There were present several thousand F_2 Bond and Victoria hybrids that had overwintered successfully, as well as over 150,000 F_2 and F_3 plants of the same hybrids from seed sown in March.

Crown rust was first found in the experimental plots on April 25, on Lee, one of the common and best types of winter oats for this region. Infections were very few; in fact, they were not abundant until the second week of May. With the continuation of excessive rains extending through April, May, and early June, crown rust assumed epidemic proportions so that by May 24 such varieties as Lee, Custis, Winter Turf, Hairy Culberson, and Oklahoma selections of Hairy Culberson × Fulghum (Winter type), showed nearly 100 per cent infection of the areas of leaves 10 or more days old. From that date until harvest—about 3 to 4 weeks later in the winter oats—as soon as the leaves became old enough to show infections, rust occupied almost all of the blades and much of the sheaths.

All Victoria hybrids, resistant and susceptible plants alike, suffered severely from crown rust. The writers' resistant Victoria hybrids, as well as some of the promising ones of Stanton and Murphy (U. S. Department of Agriculture) were so completely spotted and rusted that, compared with varieties of the Red Rustproof group, the superiority of the Victoria rust-resistant plants remains to be shown, so far as yield, weight, or other desirable character is concerned.

As a whole, even the most resistant plants—judged by the presence of minute pustules or the complete absence of pustules on the lowermost leaves and the presence of necrotic and chlorotic spots—showed numerous pustules on the upper leaves during heading, many of the pustules being moderately large and yielding a 2 to 3 type reaction. In this region Vic-

toria hybrids that show a high degree of resistance in the seedling stage become more susceptible as the plants advance in growth and maturity.

In contrast to the appearance of all Victoria hybrids under field conditions and to the Bond hybrids grown in the greenhouse, the Bond hybrids

FIG. 1. Comparison between 2 leaves each of Bond, Victoria, resistant Bond hybrid, and resistant Victoria hybrid (left to right), in amount of rusting and spotting. Leaves gathered June 16, 1938, at Fayetteville, Arkansas. Note the much greater number of pustules and chlorotic-necrotic spots on Victoria and its hybrid than on Bond and its hybrid, typical of reactions in 1938.

in the field in 1938 offer much encouragement, so far as crown rust resistance is concerned (Fig. 1). Even up to the late dough stage, none of the Bond hybrids, resistant to race 1 in the winter-oat plots, showed as much as 5 per cent of the leaf area infected with race 45. In the spring-oat plots there were a few instances in which this race was present in such abundance as to interfere with the identification of plants resistant to race 1. In by far the largest number of cases there was no difficulty in detecting resistance to race 1 in these spring oats, since race 45 was present in a very limited number of infections, in contrast with plants susceptible to race 1 which showed fully 90 to 100 per cent of the leaf area infected at heading. In addition, the abundant spotting of leaf areas in Victoria hybrids reacting to race 1, was absent in Bond hybrids.

There are 2 possible explanations for the difference in the response between greenhouse and field-grown Bond hybrids when subjected to race 45 of crown rust. 1. This race makes its appearance in the field in northern Arkansas considerably later than race 1. 2. Bond hybrids escape race 45 in much the same manner that Red Rustproof oats escape this race and race 1 in the field. In the greenhouse the latter show abundant infections and a completely susceptible reaction to both races in the seedling stage, as well as in the later stages, a fact previously noted by several investigators.

It is conceivable that if Bond hybrids were grown extensively in the South, race 45 might become as destructive as race 1 now is. However, the fact that race 45 was found in at least several collections in this region in 1937 and as late as November on potted oats placed outdoors during the summer, and the further fact that both fall- and spring-sown Bond hybrids showed a much reduced amount of infection with race 45 in 1938, compared with plants susceptible to race 1, suggest that race 45 on such hybrids is not much more menacing in Arkansas than race 10 of *Puccinia graminis avenae* is to the Richland oat and its hybrids in the Middle West. However this may be, in the presence of the severe crown-rust epidemic of 1938, Bond hybrids appeared to be far more promising than those of Victoria in their reaction to the races of rust prevalent in northern Arkansas. This duplicates reactions noted on the parent varieties Bond and Victoria in 1935, the other recent year in which crown rust was present in this region in epidemic proportions.

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THE SEPARATION OF PLANT VIRUSES BY CHEMICAL MEANS[1]

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INTRODUCTION

The differentiation of plant viruses has received considerable attention in recent years and a number of distinct viruses and strains have been described. The criteria used have been based largely upon symptomatology, differential hosts, selective insect transmission, and physical properties. Obviously it is not only desirable to distinguish these viruses but to separate or isolate them at will from other viruses with which they may sometimes be associated. Such mixtures are fairly common in nature and in accidental contaminations in experimental work. Separation is, in some instances, relatively simple when based upon the known criteria; however, in other cases it may be extremely difficult or impossible. For example, it may be possible to isolate one component of a mixture by thermal inactivation or by means of a susceptible host but especially difficult or time consuming to isolate the other component.

The differential action of chemicals upon viruses may offer many possibilities in virus separation due to the great variety of kinds and concentrations of chemicals available. A survey of this field has been made with the hope that chemicals might be found that would prove especially useful for the purpose in mind. The results have been promising to the extent that it has definitely been established that chemicals have specific action on viruses. In several instances both components of virus complexes have been isolated by differential tolerance to chemical substances. The data and conclusions of this investigation are presented in this paper.

REVIEW OF LITERATURE

The inactivating action of toxic chemicals upon plant viruses has been investigated intermittently since the work of Koning (12) and Beijerinck (3) in 1899. The purpose of these early investigations was largely to secure some clue as to the nature of the viruses. Allard (1) was the first to make a special study of the inactivation of viruses by chemicals. He treated the virus of tobacco mosaic with a wide variety of germicides, salts, acids, etc., and concluded that this virus was remarkably resistant. These earlier experiments were all with the highly tolerant virus of ordinary tobacco mosaic. More recently other plant viruses have been shown to be much less resistant to chemicals than the tobacco-mosaic virus. Unfortunately, the

[1] This investigation was conducted under allotments from the University of Wisconsin Research Fund. The paper resulting therefrom, and here presented, formed part of a thesis presented to the Graduate School of The University of Wisconsin in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

The writer is indebted to Dr. James Johnson for advice and criticism during the work and preparation of the manuscript.

results of the different investigators are not readily comparable because a standard technique has not been used. It is impossible, therefore, from the available data, to draw satisfactory conclusions concerning the actual tolerance of the different viruses to the chemicals tested.

Little has been done on the actual separation of mixtures of viruses by chemicals. Johnson (8) earlier expressed the belief that inactivation by chemicals would be useful in separation and classification of viruses. He treated many potato and tobacco viruses with alcohol and nitric acid (9) and thereby showed that differentiation, at least to some extent, was possible by this method. A few cases have been reported where one component of a virus mixture has been isolated by chemical inactivation. Shapovalov (13) treated the virus combination causing tomato streak (*i.e.*, ordinary tobacco-mosaic virus and latent potato virus) with a wide variety of chemicals. He was able with some constancy to isolate the ordinary tobacco-mosaic virus, but he found the isolation of the latent potato virus much more difficult and entirely unpredictable with the chemicals tested. He divided the chemicals into 3 groups according to their effect upon the viruses as follows: (a) those like the bile salts and boric acid, which had little or no effect upon either virus; (b) those having a strong inactivating effect upon both viruses, such as copper sulphate and sulphurous acid; and (c) those that were good inactivators but had no special affinity for either virus, *e.g.*, nickel sulphate and cobalt sulphate. This latter group was found to behave erratically, with the result that either virus might be inactivated first. Folsom and Bonde (5) treated the potato rugose-mosaic virus complex with several chemicals, but did not observe differential action or secure separation of the viruses. Freeman (6) has reported the separation of the latent mosaic virus from the potato rugose-mosaic virus complex by changing the hydrogen-ion concentration.

Stanley and Wyckoff (15) found that the tobacco-mosaic virus was more tolerant to high hydrogen-ion concentrations than the tobacco ring-spot virus, but that the latter may be more tolerant to low hydrogen-ion concentrations. It is not known whether separation by this method has been accomplished. Gratia and Manil (7) and Chester (4) have suggested that neutralization reactions of specific immune sera may be useful in the separation of virus mixtures. Stanley and Wyckoff (15) claim to have isolated the tobacco ring-spot virus from a mixture with the tobacco-mosaic virus by neutralizing the latter with specific immune serum. The author has recently published a preliminary note (2) in which the viruses of cucumber mosaic and potato ring-spot were each reported to be isolated by chemical means from a mixture. Further details of this and other separations are given in this paper.

MATERIALS AND METHODS

The viruses used in the present studies were those of ordinary tobacco mosaic (*tobacco virus 1*), ordinary cucumber mosaic, tobacco ring spot, potato ring spot, potato veinbanding, and tobacco streak. These viruses

were selected because of their availability and the wide range of physical properties they represent.

Infective extracts for treatment were obtained by extraction from diseased tobacco plants (*Nicotiana tabacum* L. var. Havana-Seed). Five to 10 plants showing typical disease symptoms were macerated with mortar and pestle and extracted through cheesecloth. The extracts were again filtered through cheesecloth to remove coarse particles of plant débris. Since the primary purpose of this investigation was to separate the viruses, and only a few of the viruses concerned have been purified to any extent, no attempt was made at their purification. The chemicals were dissolved in distilled water at twice the concentrations to be tested and then mixed with equal quantities of infective plant extract. Usually 2 cc. of the extract were mixed with 2 cc. of the chemical solution in a test tube. This gave the desired concentration of the chemical and, at the same time, diluted the virus by one half. The preparations were then held in a water bath at 20–22° C. for 1 hour, after which 1 cc. samples from each were pipetted into evaporating dishes and 50 cc. portions of distilled water were added. This procedure brought all the samples of virus extract to a dilution of 1–100 and diluted the most toxic substances sufficiently to prevent chemical injury on the inoculated plants.[2] The infectivity of each treated extract was determined by inoculation of 5 small tobacco plants (*N. tabacum* L. var. Havana-Seed) by rubbing the leaves with cheesecloth saturated in the treated preparation.

The chemicals employed represent a wide variety of compounds as follows: salts of heavy metals, heavy metal-organic compounds, phenolic compounds, oxidizing agents, reducing agents, acids, and bases. The effect of 35 chemicals that were tried on viruses are reported in this paper. Many other compounds were tried in a preliminary manner, but their use was discontinued because of inconsistent behavior. A special attempt was made to select fairly stable compounds that were known to have antiseptic properties. Ordinary laboratory grades of chemicals were used, most of them being of analytical grade, but many compounds were commercial preparations as obtained from pharmaceutical houses. The strengths of the chemical solutions are given in percentage on a weight-volume basis.

EXPERIMENTAL RESULTS

Chemical Inactivation and Tolerance of Single Viruses

The relative toxicity of different chemicals may be determined quite readily where the viruses used produce local lesions on certain hosts. With other viruses, however, it becomes a laborious task because of the number of plants required to yield significant figures. Therefore, quantitative determinations of the relative inactivating influences have not been attempted in this investigation. A chemical or a chemical concentration was not considered useful for the present purposes unless it gave complete inactivation at

[2] Preparations containing tobacco-streak virus were diluted 1–10 because of the low tolerance to dilution of this virus.

a concentration not too toxic to the inoculated host tissue following a reasonable dilution for the virus. The value of a chemical or a concentration also was determined by the maximum solubility in water, as well as by its previously known lethal action on microorganisms.

TABLE 1.—*A list of chemicals tested on the six plant viruses used in this investigation, but found insufficiently toxic to cause complete inactivation under the conditions of the experiments*

Chemical	Maximum conc. tried; per cent	Approx. pH of treated extract[a]	Reason for not trying higher concentrations[b]
"Mercury oxycyanide"[c]	1.0	7.5	C
Copper acetate	1.0	4.8	B
Zinc sulphate	10.0	4.6	D
Nickel chloride	10.0	4.5	D
Nickel nitrate	10.0	4.5	D
Protargol[c]	1.0	8.2	D
Collargol[c]	1.0	8.2	D
Flumerin[c]	1.0	8.9	C
Merthiolate[c]	1.0	7.5	B
Xeroform[c]	½ saturation	7.1	C
Bismarsen[c]	1.0	7.7	D
Sulpharsphenamine[c]	1.0	7.3	D
Catechol	5.0	6.4	B
Protocatechuic acid	1.0	4.9	C
Pyrogallic acid	5.0	6.9	D
Bile Salts—Fairchild[c]	10.0	7.3	D
Safranin	1.0	6.0	B.
Methyl orange	0.5	7.2	C
Ethyl alcohol	50.0	6.1	E
None (check)		6.4–6.9	

[a] pH measurements were made with the glass electrode method.
[b] B. Higher concentrations gave burning of leaves. C. Maximum solubility reached. D. Concentration attained that should display toxicity on basis of known antiseptic properties. E. Concentration limited by method of experimentation.
[c] Trade name.

Undoubtedly, many of the chemicals listed in table 1 will inactivate viruses, but they do not completely inactivate any of those studied here under the conditions of the experiments. The antiseptic power of mercury oxycyanide (51.7 per cent to 56.0 per cent $Hg(CN)_2$ + 44.3 per cent to 48.0 per cent HgO) is claimed to be greater than that of mercuric chloride; however, it does not react with albumins so readily as the latter. It is of interest to note here that it has little toxic effect upon viruses as contrasted to mercuric chloride (Tables 1 and 2). Protargol (a brand of strong silver protein) and collargol (colloidal silver and silver oxide) are recognized as weak antiseptics, and here appear to have very little if any inactivating influence upon viruses. Flumerin (disodium-2-hydroxy-mercurifluorescein) is a relatively weak antiseptic and did not have significant effect upon the viruses. Merthiolate (sodium ethyl mercurithiosalicylate) is a potent germicide that does not precipitate proteins and has proved of little value as a virus inactivator in concentrations that do not injure the inoculated plant. Xeroform (a basic bismuth tribromphenate of variable composition with mild antiseptic

properties) and 2 compounds containing trivalent arsenic (sulpharsphenamine and bismarsen) were ineffective as virus inactivators. Two phenolic compounds (protocatechuic acid and catechol) were not sufficiently toxic in the concentrations used to give significant inactivation. Pyrogallol, a reductant, was a weak inactivator; its strength as a reducing agent is, however, somewhat impaired by acid solutions. Since the extracts when treated with pyrogallol gave a test of approximate pH 5.0, this may account for its weak action. The bile salts (Bile Salts—Fairchild, a mixture of equal proportions of sodium glycocholate and sodium taurocholate) are reported to have mild antiseptic action; they appear here, however, to be poor virus inactivators. Safranin was unsatisfactory in this study because it is extremely injurious to plant foliage. Ethyl alcohol has been reported in many cases as a virus inactivator; its action, however, is too slow to be of value in experiments where 1 hour is the standard time for treatment.

TABLE 2.—*A list of chemicals found to be good virus inactivators, and the minimum concentration of each required to completely inactivate the respective viruses*[a]

Chemical	Approx. pH of treated extract[a]	Virus used and conc. of chemical (per cent)					
		Tobacco mosaic	Cucumber mosaic	Tobacco ring-spot	Potato ring-spot	Potato vein-banding	Tobacco streak
Mercuric chloride	5.7	3.0+[b]	0.8	0.1	0.5	0.6	0.1
Silver nitrate	5.5	4.0	2.0+	1.0	0.5	1.0	1.0+
Copper sulphate	3.5	6.0+	1.8	2.0	2.5	1.0	0.2
Lithium carbonate	10.5	1.0+	1.0[c]	1.0	1.0+	1.0	1.0
Stannic chloride	2.2	1.1	0.7	0.4	0.5	0.5	0.5
Mercurochrome[d]	8.2	2.0+	0.5	0.5	2.0+	0.5	0.5
Phenol	6.2	5.0[c]	5.0	5.0[c]	5.0	4.0	2.0
Tannic acid	6.3	5.0+	0.5	0.5	0.5	1.5	0.2
Formalin[d]	6.8	50.0+	1.0	1.0	1.0	0.5+	1.0
Oxalic acid	3.5	2.5+	0.6	1.0	1.0	0.6	0.4
Picric acid	5.9		0.6	0.4	0.6	0.4+	0.4+
Nitric acid	1.5	2.0	0.6	0.4	0.9	0.3	0.5
Sodium hydroxide	11.0	0.3	0.2	0.1	0.3	0.2	0.1
Sodium permanganate	8.7	1.5[c]	0.5	0.1	1.0	0.5	0.2
Potassium permanganate	8.7	1.5	0.4	0.4	0.8	0.2	0.2
Potassium dichromate	6.5	1.0+	0.6	0.4	0.7	1.0+	0.2
None	6.4–6.9						

[a] In 1 hour at 20° C. pH measurements were made with the glass-electrode method.
[b] Plus sign indicates inactivation not complete.
[c] Virus was usually, but not always, completely inactivated at the concentration given.
[d] Trade name.

The chemicals listed in table 2 are all good inactivators for 1 or more viruses at the concentrations given, which are the lowest reliable percentages for complete inactivation. As may be expected, some variation exists as to the concentrations required for complete inactivation of individual viruses. The minimum concentration necessary to give complete inactivation is based upon at least 3 trials. In no trial has the required concentration of a chemical been greater than given in table 2. It is believed that the concentra-

tions shown in this table represent in a general way the relative tolerance of the different viruses to the chemicals listed.

No particular types of chemicals used (Table 2) seem to be outstanding in value for virus inactivation, unless acids and bases are so considered. The concentrations of acids and bases reported in this table are quite high and the ranges in the resulting pH are extremely wide. These chemicals all possess germicidal properties and are all capable of denaturing proteins. The mode of action of the chemicals upon the viruses, however, must necessarily remain in doubt as long as the nature of viruses remains in doubt. The treatment of the viruses in unpurified extracts further obscures the nature of the inactivation. However, it is obvious from a casual examination of table 2 that it should be feasible to destroy some viruses in mixtures by chemical treatment without completely destroying others.

Chemical Inactivation and Tolerance of Virus Mixtures

Previous workers have characterized viruses by their tolerances to chemicals in general, possibly with the assumption that the viruses fall into a definite sequence as to their tolerances to all chemicals. This conception is not without foundation, as will be evidenced by an examination of table 2. Tobacco mosaic is outstanding in its tolerance to chemicals and has been recognized as such for many years. In the present investigation, over half the inactivating chemicals were not toxic enough to completely inactivate this virus and, where complete inactivation did occur, the concentrations required were relatively high. A disappointing feature of this study has been the failure, with one exception to be mentioned later, to find substances to which the ordinary tobacco-mosaic virus would prove to be more intolerant than certain other viruses. The tobacco streak virus may be cited as an example of the other extreme in regard to chemical tolerance. In table 2 the tobacco streak virus is shown to have a comparatively low tolerance to chemicals. The other 4 viruses, namely, tobacco ring-spot virus, cucumber-mosaic virus, potato ring-spot virus, and potato veinbanding virus do not differ strikingly, as a whole, in their chemical tolerance, but show significant differences in response to specific chemicals.

Cucumber-mosaic Virus and Potato Ring-spot Virus Mixture. A comparison of the viruses of cucumber mosaic and potato ring-spot in table 2 will show that the former is more tolerant to mercuric chloride, silver nitrate, and stannic chloride, while the latter is more tolerant to copper sulphate, lithium carbonate, mercurochrome, oxalic acid, nitric acid, sodium permanganate, potassium permanganate, and potassium dichromate. The significance of some of the differential tolerances of these 2 viruses as shown in table 2 is still in some doubt; however, repeated tests of several of these compounds have been made with fairly consistent results.

The cucumber-mosaic virus and the potato ring-spot virus are somewhat similar in their physical properties. The thermal inactivation points are very close, and both have a maximum tolerance to dilution of about 1–10,000

when taken from tobacco. They are, however, separable by insect transmission, aging *in vitro,* and by differential hosts. The symptoms caused by the 2 viruses are quite distinct. The potato ring-spot symptoms on tobacco are characterized at first by small necrotic spots, surrounded by a variable number of necrotic rings. About 7 days after inoculation, systemic symptoms appear in the upper leaves and consist of irregular necrotic lines and concentric rings. Ordinary cucumber-mosaic virus manifests itself on tobacco by the production of chlorosis and some leaf distortion. When the 2 viruses are present simultaneously in tobacco, the resulting symptoms are very striking (Fig. 1). In late stages all the leaf tissue and much of the stem are enveloped in a systemic necrosis. These later symptoms are particularly severe under high air-temperature conditions.

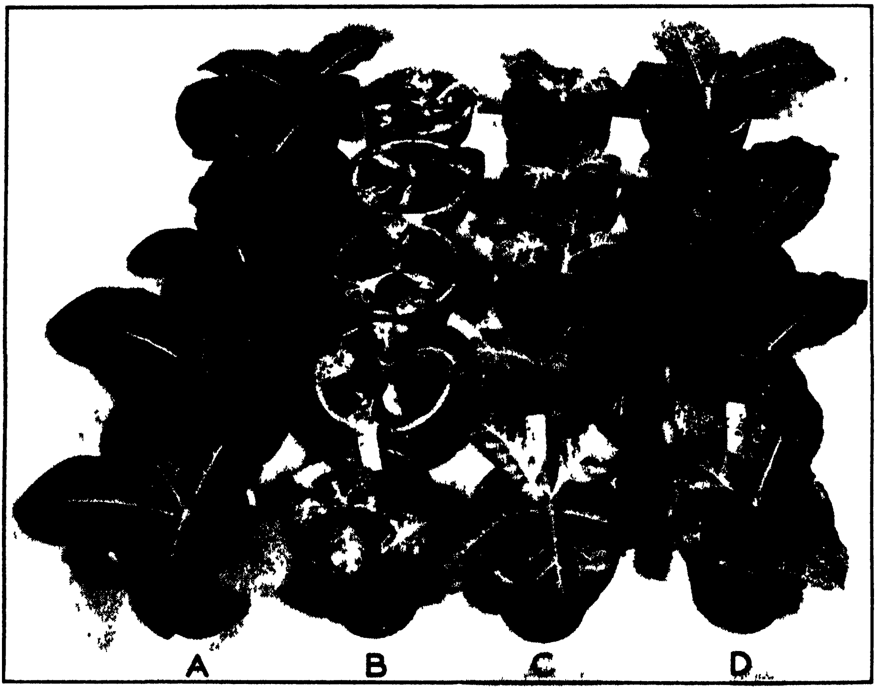

FIG. 1. The separation of the viruses of cucumber mosaic and potato ring spot from a mixture. A. Healthy, noninoculated controls. B. Plants inoculated with the nontreated virus mixture, showing severe stunting and necrosis. C. Plants inoculated with the same mixture treated with 0.3 per cent silver nitrate and showing typical cucumber-mosaic symptoms only. D. Plants inoculated with the same mixture as in B, but treated with 1.0 per cent lithium carbonate and showing typical potato ring-spot symptoms only.

Plant extracts containing cucumber-mosaic and potato ring-spot viruses were prepared as formerly and then combined, giving a mixture of the 2 viruses. This preparation was then treated with a series of concentrations of each of the following chemicals: silver nitrate, mercuric chloride, copper sulphate, lithium carbonate, and potassium permanganate. The conditions

and methods of the experiment were similar to those of the previous section involving inactivation of single viruses.

TABLE 3.—*The separation of the cucumber-mosaic virus and the potato ring-spot virus by chemical treatment from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with cucumber-mosaic only	with potato ring-spot only	with neither virus
Silver nitrate	0.1	5	0	0	0
	0.2	1	4	0	0
	0.3	0	5	0	0
	0.4	0	5	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
Mercuric chloride	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	5	0	0	0
	0.4	5	0	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
	0.7	0	5	0	0
	0.8	0	0	0	5
Potassium permanganate	0.2	5	0	0	0
	0.3	0	0	5	0
	0.4	0	0	1	4
	0.5	0	0	0	5
	0.6	0	0	0	5
Copper sulphate	1.6	3	0	2	0
	1.7	0	0	0	5
	1.8	1	0	1	3
	1.9	0	0	1	4
	2.0	0	0	2	3
Lithium carbonate	1.0	0	0	5	0
None (check)		5	0	0	0

A typical set of results appears in table 3. Silver nitrate and mercuric chloride completely inactivated the potato ring-spot virus at concentrations that left the cucumber-mosaic virus infectious. Potassium permanganate, copper sulphate, and lithium carbonate behaved in the opposite manner, being more toxic to the cucumber-mosaic virus with the result that the potato ring-spot virus only remained infectious. Figure 1 shows how striking these results appear on the greenhouse bench. Here is shown the isolation of cucumber-mosaic virus from the mixture by treatment with silver nitrate and the isolation of the potato ring-spot virus by treatment with lithium carbonate.

With the exception of the copper sulphate treatment, this experiment has been repeated 10 times. The isolation of potato ring-spot virus with copper sulphate has been repeated only twice, but with similar results in each case.

The conclusion that the viruses in question have been isolated has been proved in many cases by successive inoculations of the isolated viruses to tobacco. In every instance where separation was apparent from the symptoms, the further inoculations confirmed the isolation. In several experiments, the infective extracts for treatment were taken from plants infected simultaneously with both viruses instead of making the combination artificially by mixing extracts from plants infected with the different viruses singly. In other experiments, the extracts were taken from plants that had been inoculated for many days with one virus and following the appearance of symptoms were inoculated with the other only a few days before extraction. In these experiments, the resulting fluctuations in concentrations of the viruses used were not sufficient to alter the results.

Potato Veinbanding Virus and Potato Ring-spot Virus Mixture. A common virus disease of potatoes, known as rugose mosaic, has been shown by Koch (11) and others to be caused by a combination of the latent potato virus and the veinbanding virus. This is a common virus mixture occurring in nature causing considerable injury to potatoes in some districts. The latent potato virus occurs universally in most of the American varieties of potatoes and, as fas as is known, is disseminated in nature solely by the vegetative propagation of the potato. It is, however, readily transmitted by mechanical means. The potato ring-spot virus used in these studies is a strain of this latent potato virus and was isolated by Koch (11). The veinbanding virus is readily transmitted mechanically or by certain aphids, has a slightly lower thermal inactivation point than the potato ring-spot virus, and is less tolerant to aging and dilution. The symptoms on tobacco caused by the veinbanding virus are a slight stunting of the plant, accompanied by a faint vein clearing and vein banding with a drooping of the leaves. The "spot necrosis" symptom on tobacco produced by a mixture of these 2 viruses has been frequently described. Typically, it is a severe stunting with necrotic streaks along the veins and necrotic spots between the veins. It is, therefore, relatively simple to distinguish between tobacco plants infected with the veinbanding virus, the potato ring-spot virus, and a combination of the 2 viruses. There are specific differences between the relative tolerances of these 2 viruses to chemicals (Table 2). The veinbanding virus is more tolerant to mercuric chloride and silver nitrate, while the potato ring-spot virus is more tolerant to most of the other chemicals listed.

The veinbanding and potato ring-spot viruses were artificially combined and treated with chemicals, as was the previously described virus mixture. As shown from the results of a typical experiment given in table 4, the veinbanding virus can be separated from the potato ring-spot virus in a way similar to that of the cucumber-mosaic virus to which it is believed by some to be related. The veinbanding virus again appears to be more tolerant to mercuric chloride than the potato ring-spot virus. Time has not permitted a test on possible separation with silver nitrate. From the data in table 2, it seems probable that the isolation could also be made by this means. The

TABLE 4.—*The separation of the potato ring-spot virus and the potato veinbanding virus by chemical treatment from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with potato-veinbanding virus only	with potato ring-spot virus only	with neither virus
Mercuric chloride	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	0	5	0	0
	0.4	0	1	0	4
	0.5	0	0	0	5
Potassium permanganate	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	5	0	0	0
	0.4	5	0	0	0
	0.5	0	0	5	0
	0.6	0	0	5	0
Sodium hydroxide	0.1	5	0	0	0
	0.2	0	0	5	0
	0.3	0	0	0	5
Nitric acid	0.1	5	0	0	0
	0.2	0	0	5	0
	0.3	0	0	0	5
None (check)		5	0	0	0

results shown in table 4 have been repeated 3 times with almost identical results. In one experiment, the inoculum was taken from plants with "spot necrosis" symptoms, *i.e.*, infected by both viruses simultaneously and the isolation of both viruses was completed by chemical means. In some cases repeated transfers were made to other tobacco plants to check the purity of the isolates. In every instance tested, the viruses isolated were found to be free from the other virus of the complex.

One attempt has been made by this method to isolate each of the viruses from artificially infected rugose-mosaic potato plants. The results have been partly negative since the latent-mosaic virus only was isolated. However, since the untreated controls yielded only 1 plant in 5 infected with the veinbanding virus, it was concluded that for some reason the veinbanding virus was not present in sufficient concentration to make the trial reliable.

Cucumber-mosaic Virus and Tobacco Ring-spot Virus Mixture. The viruses of cucumber mosaic and tobacco ring-spot in combination have not been reported as associated in nature in the production of any diseases. They, however, form an ideal combination for a study in which the aim is to investigate the possibilities of the separation of viruses by chemical inactivation. The above viruses have quite similar points of thermal inactivation and tolerance to dilution, but are separable by aging, insect transmission, and the use of differential hosts. In combination, they do not produce such

striking symptoms as do the 2 previously studied complexes, each virus continuing to produce characteristic symptoms, so the presence of either is not masked. Generally, the plants are stunted more than when infected by either virus alone.

As shown in table 2, the cucumber-mosaic virus is slightly more tolerant to most chemicals than the tobacco ring-spot virus. Phenol and oxalic acid, however, appear to be less toxic to the latter virus than to that of cucumber mosaic. Consequently, the combination of the 2 viruses was treated with phenol to inactivate the cucumber-mosaic virus and with mercuric chloride to inactivate that of ring spot. The experiment was performed, using methods similar in every respect to those of the 2 foregoing experiments.

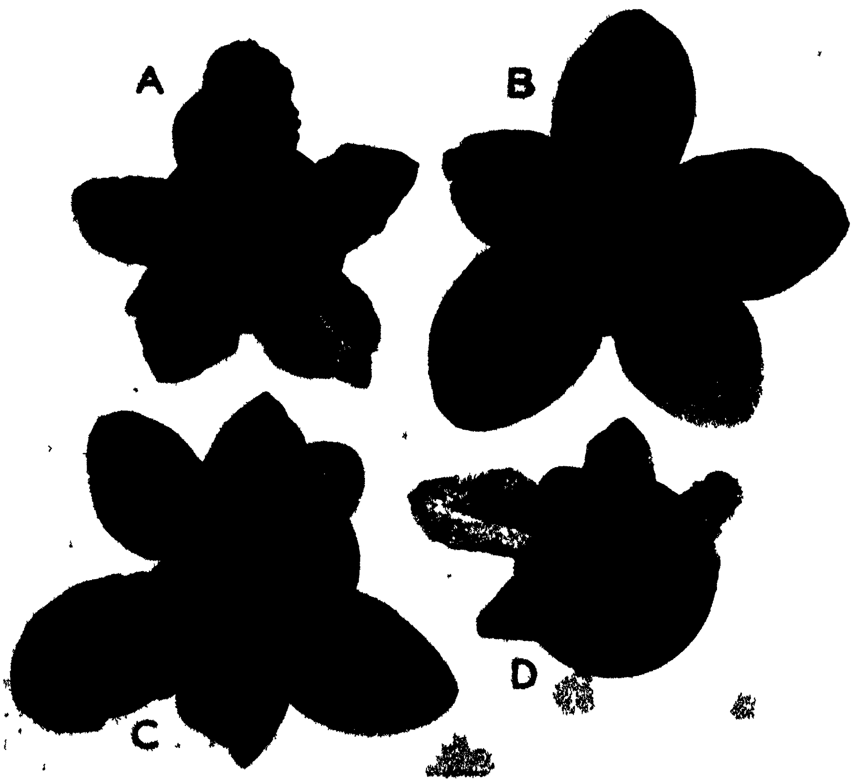

FIG. 2. The separation of the viruses of cucumber mosaic and tobacco ring spot. A. Plant inoculated with an extract containing both viruses, treated with 0.1 per cent mercuric chloride. Infection with cucumber-mosaic virus only has developed. B. Healthy, noninoculated control. C. Plant inoculated with the same mixture as A, but treated with 4.0 per cent phenol. Symptoms of tobacco ring spot only are evident. D. Plant inoculated with the nontreated extract containing both viruses.

The results of a typical experiment are given in table 5. As expected, the combination was resolved into its 2 component viruses (Fig. 2). In 4 trials, 4.0 per cent phenol completely inactivated the cucumber-mosaic virus, leaving the tobacco ring-spot virus infective. In a fifth trial, when 5 plants

TABLE 5.—*The separation of viruses of cucumber mosaic and tobacco ring spot by phenol and mercuric chloride from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with cucumber-mosaic only	with tobacco-ring-spot only	with neither virus
Mercuric chloride	0.4	0	5	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
	0.7	0	5	0	0
	0.8	0	0	0	5
Phenol	1.0	5	0	0	0
	2.0	5	0	0	0
	3.0	5	0	0	0
	4.0	0	0	5	0
	5.0	0	0	0	5
None (check)		5	0	0	0

were inoculated with an extract treated with 4.0 per cent phenol, 3 plants were infected with tobacco ring spot only, 1 plant with cucumber mosaic only, and 1 with both viruses. In most instances, 5.0 per cent phenol completely inactivated both viruses when they were in combination. Occasionally, however, the tobacco ring-spot virus remained infective. This result with phenol does not agree perfectly with the results given in table 2, where 5 0 per cent phenol is shown not to completely inactivate either virus. The values given in table 2 may be slightly high because of the occasional failures of certain chemicals to completely inactivate at concentrations that ordinarily produce complete inactivation. In some cases, it is also very probable that the viruses in combinations do not attain their usual concentrations and for this reason may be less tolerant to the treatment given. The concentrations, for example, are reduced one-half at the outset, when the combinations are obtained, by mixing extracts from plants with both viruses.

Tobacco-mosaic Virus and Tobacco Ring-spot Virus Mixture. For many years investigators have concluded that the tobacco-mosaic virus is much more tolerant to chemicals than any other recognized plant virus. Consequently, the writer considered the isolation of other viruses from a mixture containing tobacco-mosaic virus by chemical inactivation as a challenge to be met in an attempt to prove the value of this method.

An examination of table 2 reveals the ordinary tobacco-mosaic virus to be much more tolerant than any of the other viruses to all the chemicals used, with the exception of phenol. Since the tobacco ring-spot virus appeared to be particularly tolerant to phenol, it was combined with tobacco mosaic virus in the ordinary manner of equal parts by volume of extract. Treatment with phenol and potassium permanganate in varying concentrations was then made, as in preceding experiments. Contrary to expectations, the tobacco-mosaic virus tolerated higher concentrations of both chemicals than did the

ring-spot virus. The concentration of a virus no doubt bears some relation to the amount of a chemical necessary to cause complete inactivation. On the basis of tolerance to dilution, the tobacco-mosaic virus is roughly 1000 times as concentrated as the tobacco ring-spot virus in ordinary fresh tobacco extract. Therefore, an effort was made to separate these viruses when more nearly in the same concentrations. An extract for treatment, therefore, was prepared that consisted of 1 part tobacco-mosaic extract and 99 parts tobacco ring-spot extract. The mosaic virus was nevertheless roughly 100 times more concentrated than the ring-spot virus, as may be observed from the results of dilution tests (Table 6). Employing the usual procedure, this mixture was treated with 0.5 per cent sodium permanganate and various concentrations of phenol.

The results of one experiment presented in table 6 indicate a complete sep-

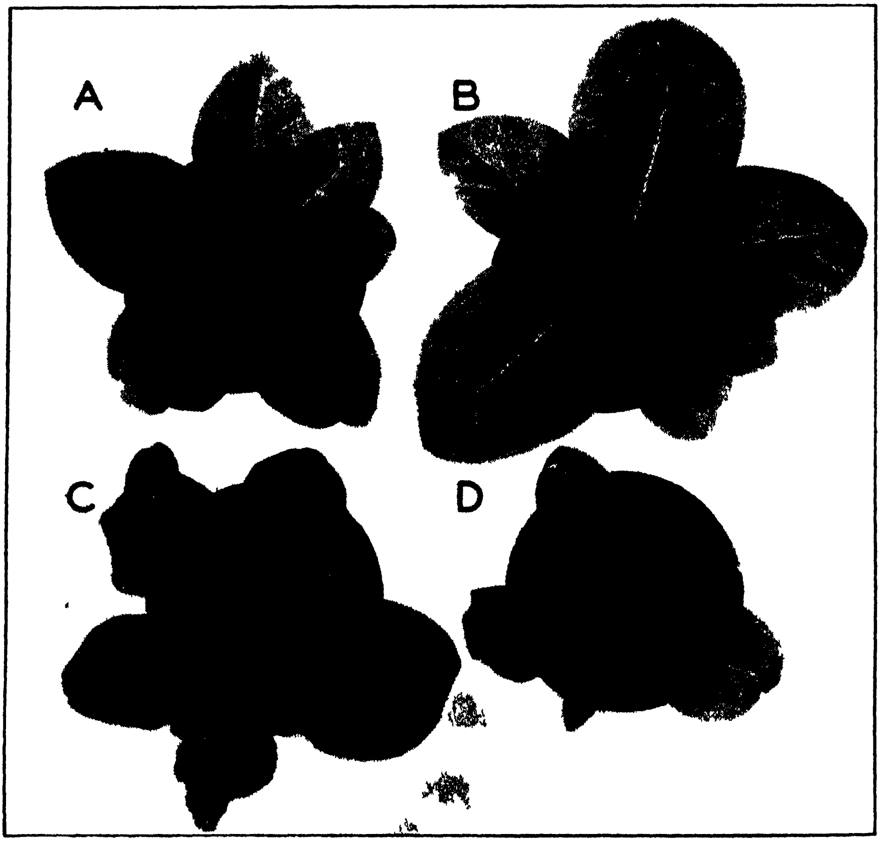

FIG. 3. The separation of the viruses of tobacco mosaic and tobacco ring spot with chemicals when the tobacco-mosaic virus has previously been diluted 1–100 (see page 904). A. Plant inoculated with an extract containing both viruses treated with 0.5 per cent sodium permanganate, showing the presence of tobacco mosaic only. B. Healthy, noninoculated control. C. Plant inoculated with the same mixture as A, but treated with 4.0 per cent phenol. Note that tobacco ring-spot symptoms only are evident. D. Plant inoculated with nontreated extract containing both viruses. Symptoms of both virus diseases are present.

aration of the 2 viruses and, consequently, a specific action by the chemicals. Sodium permanganate inactivated the ring-spot virus without completely

TABLE 6.—*The separation of viruses of ordinary tobacco mosaic (diluted 1–100) and tobacco ring spot with phenol and sodium permanganate from a mixture of the viruses in plant extract, together with the effect of dilution upon the nontreated extract*

Chemical	Dilution	Conc. chem. per cent	Number of infected plants out of 5[a] inoculated following treatment of virus combination as shown			
			with both viruses	with tobacco-ring-spot virus only	with tobacco-mosaic virus only	with neither virus
Phenol		1.0	5	0	0	0
		2.0	5	0	0	0
		3.0	5	0	0	0
		4.0	0	5	0	0
		5.0	0	0	2	3
Sodium permanganate		0.5	0	0	5	0
Water	1–10		10	0	0	0
	1–100		8	0	2	0
	1–1000		0	0	10	0
	1–10,000		0	0	5	5
	1–100,000		0	0	2	8

[a] In case of water dilutions 10 plants were inoculated.

destroying the tobacco-mosaic virus, whereas 4.0 per cent phenol inactivated the tobacco-mosaic virus without completely destroying the ring-spot virus. The comparative symptoms are illustrated in figure 3, and a check made on the isolates by repeated inoculation to tobacco and the *N. glutinosa-N. tabacum* hybrid verified the purity of the respective viruses. This experiment has been performed 3 times with almost identical results in each trial. Concentrations of phenol below 4.0 per cent have not completely inactivated either virus. In one experiment, one plant of the group of 5 inoculated with the combined extracts treated with 4.0 per cent phenol was infected with tobacco-mosaic virus. Tobacco ring-spot symptoms occurred in only one plant of those inoculated with the extract containing 5.0 per cent phenol. Without exception, tobacco mosaic has appeared in from 1 to 3 plants in each set inoculated with the extracts treated with 5.0 per cent phenol. That is, 5.0 per cent phenol gave less inactivation of the tobacco-mosaic virus than 4.0 per cent phenol. The reason for this unusual result is obscure, although a possible explanation may be offered. When crude tobacco extract is treated with 5.0 per cent phenol, considerably more coagulation and clumping occurs than in the preparations treated with 4.0 per cent phenol. Since the tobacco-mosaic virus is roughly 100 hundred times more concentrated than the ring-spot virus in these extracts, it is not improbable that the chances for occlusion of the mosaic virus in the clumps is much greater. When the virus is surrounded by a mass of coagulated and precipitated material, it may not be readily accessible to the action of phenol.

Only 4 virus mixtures have been treated with chemicals; therefore, this work must be considered as of a preliminary nature. A sufficient number of repetitions, however, have been made to give assurance that at least in these virus mixtures separation by chemical inactivation is a reliable method.

DISCUSSION

The action of toxic substances has been widely studied in microbiology and the value of a large variety of chemicals for differential as well as for disinfection purposes is especially obvious. An attempt to utilize these principles in virus studies appeared logical, although at first obscure. The results secured from this study, nevertheless, are of considerable interest and suggest many possibilities for further investigation.

The repeated isolation of all the viruses in the 4 combinations studied indicates that separation by chemical specificity is feasible and may become a useful adjunct to the established methods of isolation. Satisfactory separation depends upon complete inactivation of all but one of the viruses present. Therefore, to be reliable, the specific behavior of chemicals must be great enough to offset factors such as variability in concentration of virus or in composition of plant extract, which might appreciably alter the tolerance of the virus to chemicals. Greater specificity probably would be found in purified virus preparations; but, until more viruses have been purified, a study of this type must be limited to the use of crude extracts.

The nature of the inactivating action and the specificity is obscure. Action of the chemical may be directly on the virus, indirect through chain reactions in the plant extracts, or possibly it may affect the susceptibility of the host to infection, as is claimed by Stanley (14) for the inactivating action of trypsin. In some cases the inactivation probably is reversible, as has been shown by Thornberry (16). Hydrogen-ion concentration seems not to be correlated with specificity, but the possibility is recognized that it might affect the toxicity of the different chemicals and thereby affect specificity. All the substances that were found to be good inactivators are protein denaturants and possess high germicidal properties. However, two potent germicides (Merthiolate and "mercury oxycyanide") were extremely weak inactivators.

It is highly desirable that future work concerning the identification and the separation of viruses by chemicals be conducted under more uniform conditions as to time and temperature of treatment. The lack of such uniformity in the past makes an adequate evaluation of previous work of this type difficult or impossible. A standard set of conditions should be adopted that falls within the working range of all the viruses. For example, a time of treatment as long as one day is generally unsatisfactory because many viruses naturally are inactivated by aging *in vitro* in that time. The writer has found the conditions used in the present experiments satisfactory, namely, 1-hour treatment at 20° C., of crude plant-virus extract with an equal volume of the chemical at double strength. It is suggested that this

set of conditions be tentatively adopted as a standard basis for future studies of a similar sort. One hour is less than the inactivation time by aging *in vitro* of practically all sap-transmissible viruses. The temperature of 20° C., is convenient to maintain in any laboratory. The final dilution (1–100) used in these studies may be high, but it is satisfactory for most viruses, and in some instances may be varied with little or no complication. The influence of host plants used as a source of the inoculum for chemical-inactivation studies may be in some doubt. Adequate data are not available to show what influence the host may have in such studies, although in a preliminary study Johnson and Grant (10) concluded that the viruses have somewhat characteristic tolerances to chemicals regardless of the host in which they are found. Since tobacco is commonly used in virus studies, probably the inoculum for treatment may best be obtained from this host whenever possible.

Many possibilities for future development of this problem are evident. For convenience of observation, only viruses giving necrotic and chlorotic types of symptoms have been used in this investigation. The writer, however, can conceive of no logical reason why all sap-transmissible viruses would not be amenable to this technique of separation. With this in mind, a study should be made to determine if chemicals of the same type have characteristic differential values, as is indicated by some results obtained in the present investigation. The greatest interest and value of studies of the type reported in this paper may lie in the eventual use of chemicals for the differentiation and separation of specific viruses not readily distinguished or isolated by other means.

The possible relation of chemical specificity to certain other problems concerning viruses is readily recognizable. The high degree of specificity for viruses noted in many insect vectors may possibly be related to this phenomenon. Chemotherapy sometimes depends almost entirely upon specific toxicity. Virus sanitation and prophylactic measures depend in some measure at least upon the specific action of chemicals. In plant-virus sanitation, the virus of ordinary tobacco mosaic is of special interest. Allard (1) reported complete inactivation of this virus with 4.0 per cent formaldehyde in 20 minutes. In the investigation here described, it was found that the strongest concentration of formalin (approx. 40.0 per cent formaldehyde) used (50.0 per cent) did not completely inactivate this virus in plant extract in 1 hour (Table 2). Other investigations have shown that Allard may have been working in part with the cucumber-mosaic virus, which at that time was not clearly differentiated from the tobacco-mosaic virus. The result obtained by Allard for the inactivation of tobacco-mosaic virus with formaldehyde agrees quite closely with the result obtained in this investigation with cucumber-mosaic virus (Table 2). These results show that the common practice of treating tobacco seedbed frames, etc., with formalin or mercuric chloride to destroy the tobacco-mosaic virus may be quite inadequate. Strong bases appear to be sufficiently toxic to warrant their trial in such sanitary measures. The application of chemical specificities to

virus problems is an open field from which many interesting and valuable developments may be expected.

SUMMARY

The inactivating effect of 35 chemicals upon the viruses of ordinary tobacco mosaic (*tobacco virus 1*), ordinary cucumber mosaic, potato ring-spot, potato veinbanding, tobacco ring-spot, and tobacco streak has been studied. The chemicals were selected on the basis of their known antiseptic properties and diverse mode of action as toxic agents.

The differential action of certain chemicals on the viruses used has been demonstrated. For example, the ordinary cucumber-mosaic virus is more tolerant to mercuric chloride and silver nitrate than is the potato ring-spot virus, but the cucumber-mosaic virus is less tolerant to copper sulphate, potassium permanganate, or lithium carbonate than is the potato ring-spot virus.

Each of the components of the following virus mixtures have been isolated repeatedly by treatment with specific chemicals: potato ring-spot virus plus ordinary cucumber-mosaic virus, potato ring-spot virus plus potato vein-banding virus, tobacco ring-spot virus plus ordinary cucumber-mosaic virus, and ordinary tobacco-mosaic virus plus tobacco ring-spot virus.

It is believed that the utilization of the differential action of chemicals on viruses may become of considerable value for purposes of their isolation and identification where other means are inconvenient or inadequate.

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OVERWINTERING OF TAPHRINA ROBINSONIANA[1]

W. WINFIELD RAY[2]

(Accepted for publication Aug. 20, 1938)

The generic limitations of *Exoascus* and *Taphrina* proposed by Sadebeck (6), based upon the presence or absence of perennial mycelium, have been accepted by many mycologists. The concept of Giesenhagen (2), merging all legitimate species of *Exoascus, Taphrina,* and *Magnusiella* in one genus, *Taphrina,* is accepted by the writer and by most workers of this generation. At present no noteworthy or practical characteristics have been discovered that justify the use of more than one genus for the species of this group of fungi.

It has been demonstrated by Mix (4) and by R. E. Fitzpatrick (1) that *Taphrina deformans* (Berk.) Tul. does not possess a perennial mycelium. Mix (3) has shown also that *T. mirabilis* (Atk.) Gies. depends upon overwintered spores for inoculum for initial infections. Both of these species overwinter, according to Sadebeck (6), by means of perennial mycelium, and were, therefore, included in the genus *Exoascus,* as understood by him.

Taphrina robinsoniana Gies. is the only species commonly affecting bracts of female catkins of *Alnus incana* (L.) Moench. in New York State (Fig. 1). For the past several years the disease has been very severe in central New York, and no trees of the great number observed have escaped some degree of infection. About mid-July the hypertrophied bracts make their appearance, and fresh material may be collected from that time until September. After the first deformed bracts appear, others retarded in their development by dry weather, appear during succeeding periods of rain.

Taphrina amentorum (Sad.) Rost., which occurs commonly in Europe, affects the bracts of the female catkins of *A. incana* and *A. glutinosa* Gaertn. just as does *T. robinsoniana.* This European species, according to Sadebeck (6), Giesenhagen (2), and others, is said to overwinter by means of perennial mycelium in the buds. Although the 2 species under discussion differ morphologically, the mycelium in both is subcuticular only and both species produce identical symptoms. Since they are similar in several respects, it might be expected that their life cycles are similar. No acceptable evidence has ever been presented to prove that *T. amentorum* actually possesses a perennial mycelium, though most discussions of it include a reference to its assumed perennial nature.

Giesenhagen (2), who named *Taphrina robinsoniana,* did not mention perennial mycelium, probably because he had only mature, dry material with which to work. Patterson (5), who confused the two species and

[1] A portion of a thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to express his appreciation and thanks to Professor H. M. Fitzpatrick, who proposed the problem and offered helpful suggestions, and to Professor H. H. Whetzel for a critical reading of the manuscript.

FIG. 1. Female catkins of *Alnus incana* showing deformation of bracts caused by *Taphrina robinsoniana*.

called all of her collections *E. amentorum* Sad., stated that the mycelium is perennial, but gave no evidence to support her statement.

Experiments have been made by the writer in 2 successive years to determine whether *Taphrina robinsoniana* does overwinter by means of perennial mycelium, or whether spores instead, are not responsible for primary infections.

HISTOLOGIC EXAMINATIONS

In early April of 1936 a number of female catkins from trees that had been badly infected the season before were collected and brought into the laboratory for examination. Some of the aments were fixed, imbedded in paraffin, sectioned, and stained according to accepted methods of cytological technique. Others were cleared in lacto-phenol solution and stained with either cotton blue or acid fuchsin. Although a large number of catkins were examined, no mycelium was found. Later in the summer the trees from which specimens had been collected for these studies were found to be practically 100 per cent diseased. On the basis of histologic evidence, *Taphrina robinsoniana* does not possess a perennial mycelium.

SPRAYING EXPERIMENTS

The effect of spraying on the control of the disease was investigated. It could be assumed that spraying will give no control, if the fungus overwinters by means of a perennial mycelium.

A series of spraying experiments was carried out during the spring of 1937. On April 25, 6 young trees in a large group were selected and received a dormant spray of lime-sulphur solution, 1:40. Application was made with a quart-size hand sprayer. On May 2 another application was made on the same 6 trees. Two nearby trees were chosen as checks. On July 19, when the asci were formed and were discharging their spores, the sprayed and check trees were examined and estimates of the percentage of infection made. No attempt was made to count the diseased catkins, for it was obvious that the sprayed trees had fewer of them than the nonsprayed checks. The percentage of infection on the checks was between 90 and 100, while the estimated percentage of infection for the 6 sprayed trees averaged 19. Improved methods in the application of the spray should give better control. The results, however, indicate that overwintered spores induce primary infections.

BAGGING EXPERIMENTS

Another experiment was conducted in which clusters of female catkins were enclosed in transparent, water-proof bags. As many as 12 and not less than 3 female catkins in a cluster were sprayed with a 1:40 lime-sulphur solution, and over each of 25 clusters was placed a bag, the top of which was twisted tightly around the branch and made secure with a cord. In addition, 5 nonsprayed clusters of female catkins were enclosed by bags. These served as checks.

The experiment was begun in April, 1937, and the bags were not opened until July 19. At that date the catkins were examined. The catkins and leaves were well developed, green, and without apparent infection in any of the 30 bags. Exposed catkins on the bagged branches were in every case heavily infected. The bags were left off the catkin clusters until August 21, when an examination showed that every catkin remained healthy.

Why the nonsprayed catkins enclosed by the bags should remain healthy also is not easily answered, but it is the writer's belief that any spores that might have been on the surface of the host parts enclosed by bags failed to find conditions favorable for germination and penetration into the bracts. If the mycelium were perennial, its development within the host should have occurred, for the catkins and leaves developed normally and did not show any ill effects of having been enclosed by the bags.

The evidence presented by the writer, *i.e.*, failure to demonstrate perennial mycelium in the buds by histologic methods, lowering of the percentage of infection by application of a dormant spray, and absence of infection when the catkins were enclosed by bags, indicate that *Taphrina robinsoniana* does not overwinter by means of a perennial mycelium.

LONGEVITY OF ISOLATES

Several facts at hand suggest that spores of *Taphrina robinsoniana* could overwinter on the surfaces of the host. Examination of overwintered, diseased bracts in the spring disclosed spores in some of the asci.

Isolations of the fungus from bracts were made in January by means of the dilution-plate method. If spores remained viable until that time, with low temperatures prevailing until the inoculation period in the spring, it is logical to assume that the spores would still be capable of causing infection.

Pure cultures, isolated by allowing spores to shoot onto agar plates from freshly collected specimens, were streaked on potato-dextrose agar slants in July and held at 24° and 27° C., respectively. Transfers to fresh tubes of media were made at regular intervals to test viability. The cultures remained alive until January at 27° C. and until March at 24° C. At these times the periodic transferring was discontinued because the media in the original cultures dried out. The ability of the fungus to withstand these higher temperatures for such long periods of time indicates that the spores could survive in nature from September until the inoculation period at normal winter temperatures.

It is possible and very probable that the spores lying free upon the surface of the buds and twigs are able to multiply saprophytically. The ability to multiply in nature would make certain the retention of life of the spore for at least the overwintering period. Even if such a multiplication did not occur, it is reasonable to expect, on the basis of the data presented, that spore viability could be maintained over winter.

SUMMARY

Histologic studies of dormant female catkins of *Alnus incana* disclosed no perennial mycelium in the tissues.

Applications of 1:40 lime-sulphur solution, as a dormant spray, reduced the percentage of infection considerably.

Female catkin-clusters enclosed in transparent, water-proof bags reached maturity without becoming infected.

Results of cultural studies suggest that spores could remain viable in nature from the time of their dispersion until the inoculation period.

The writer concludes from his investigations that *Taphrina robinsoniana* does not overwinter by means of perennial mycelium in the tissue of bracts of female catkins of *Alnus incana*.

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STUDIES ON PEA VIRUS 1

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INTRODUCTION

Pea virus 1 differs from other viruses producing diseases in legumes in undergoing an incubation period of about 12 hours before it can be transmitted by the vectors *Macrosiphum pisi* Kaltenbach and *M. solanifolii* Ashmead (*M. gei* Koch) (3, 5). Although the virus is difficult to transmit by the ordinary rubbing method, transmission has been secured in a high percentage of the plants inoculated by following the method of Rawlins and Tompkins (7), using carborundum powder as an abrasive. Transmission by this method has made it possible to study some of the physical properties of the virus *in vitro*. In addition, studies have been made on the efficiency of mechanical transmission as compared with aphid transmission, on the effect of exposing viruliferous aphids to high temperatures, and on the occurrence of distinct strains of the virus. The purpose of this paper is to present the results of these studies.

EXPERIMENTS

Transmissibility of Pea Virus 1 from Plants Inoculated by Aphids and from Plants Inoculated Mechanically

Preliminary experiments indicated that pea virus 1 could be transmitted more readily from aphid-inoculated plants than from mechanically inoculated plants. In early trials virus from *Vicia faba* L. plants showing pronounced symptoms of disease about 3 weeks after inoculation by aphids was used. Of 60 plants inoculated by rubbing, 31 became diseased. Subinoculations were then made from these diseased plants after an equal period of time to determine whether the virus could be maintained mechanically by serial passage in *V. faba*. No infection was obtained in the plants sub-inoculated. No constant differences had been noticed in the symptoms of disease produced in the aphid-inoculated plants when compared with those inoculated mechanically, except perhaps a tendency for the symptoms to be more severe and to spread more rapidly in the aphid-inoculated plants.

To determine definitely whether first symptoms of disease appear later in plants inoculated mechanically than in plants inoculated with colonies of aphids, an experiment was conducted in which the inoculated plants were observed daily for first symptoms of disease. Two sets each containing 30 plants were inoculated. One set was inoculated by exposure for 2 days to pea aphids that had fed on diseased plants, 25 aphids being placed on each plant. The other set was inoculated mechanically with extracts from the same diseased plants on which the aphids had fed. Of the 30 plants inoculated by aphids, 29 became diseased, while of the mechanically inoculated plants, 19 became diseased. Of the 29 plants infected by aphids, first symp-

toms were observed in 4 to 5 days in 27 of the plants. First symptoms appeared on 1 plant on the 6th day and on 1 plant on the 7th day. Of the 19 plants infected by mechanical inoculation, 3 showed symptoms in 5 days, 6 in 6 days, 3 in 8 days, 2 in 9 days, 2 in 10 days, 1 in 12 days, 1 in 14 days, and 1 in 16 days. This experiment demonstrated that first symptoms of disease appear somewhat more promptly in aphid-inoculated plants than in mechanically inoculated plants.

In an effort to explain the difference in transmissibility, experiments were planned to compare transmission from aphid-inoculated plants with that from plants inoculated mechanically under conditions as nearly identical as possible. In one experiment pea aphids were fed for 2 days on 5 diseased *Vicia faba* plants that had been inoculated by aphids 75 days previously. The aphids were then removed and the juice extracted from the diseased plants. One set of 20 *V. faba* plants was then inoculated by exposure of each plant to 25 aphids for 2 days, while another set of 20 was inoculated mechanically with juice extracted from the diseased plants. Of the 20 plants exposed to aphids, all became diseased, while 14 of the 20 inoculated mechanically became diseased. Ten diseased plants were selected from each lot and were tested in pairs. The first test was made 17 days after inoculation of the plants. In tests with aphids a plant from each of the lots was placed in each of two adjacent cages in the greenhouse. About 150 pea aphids were fed on each of the diseased plants for from 24 to 48 hours to insure proper incubation of the virus in the insects. On removal of the aphids the plants were crushed and the juice from each was rubbed on the leaves of 5 small plants. Five small plants were also exposed for at least 24 hours to each lot of aphids removed from the diseased plants. This procedure was repeated for the tests with each pair of diseased plants at various intervals after inoculation. The results are shown in table 1. No

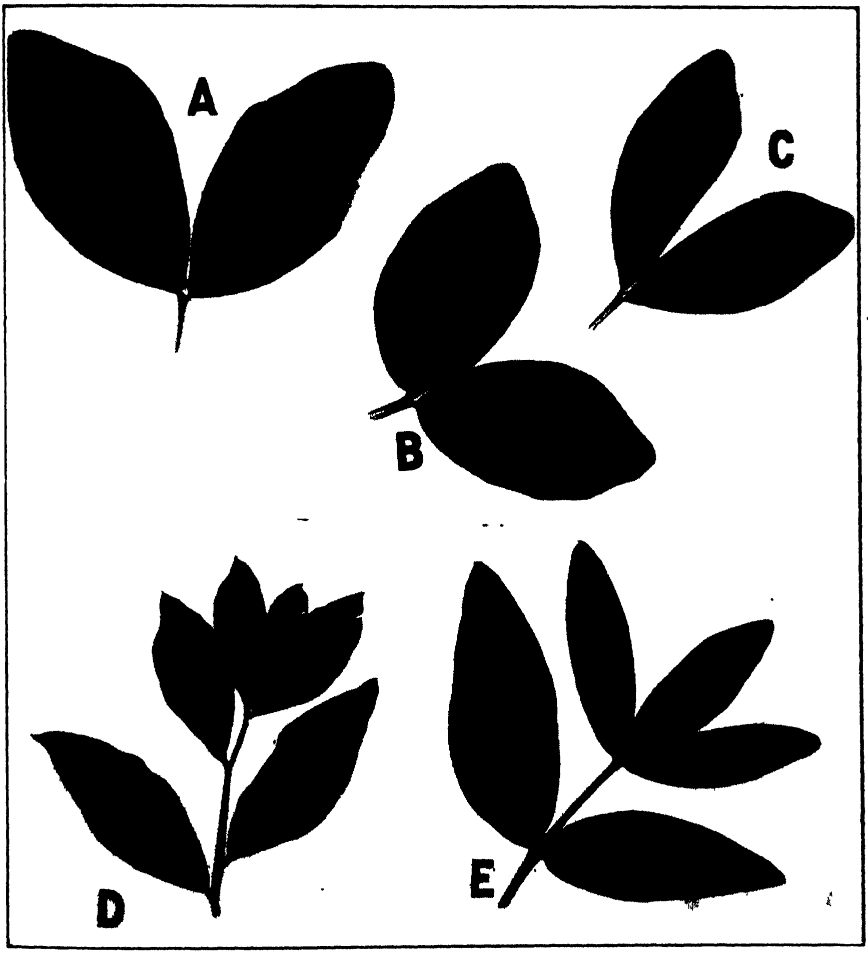
TABLE 1.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

Test number	No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
		By insects	By rubbing	By insects	By rubbing
1	17	3[a]	5	0	0
2	22	5	2	0	0
3	26	5	5	0	0
4	30	5	5	0	0
5	33	5	5	0	2
6	35	5	5	0	0
7	36	5	5	0	0
8	40	2	5	0	2
9	42	5	5	0	2
10	45	5	5	3	5
		45	47	3	11

[a] Five *Vicia faba* plants were used in each test.

infection was obtained either by aphids or by mechanical inoculation from mechanically inoculated plants in the first 4 tests, while transmissions were obtained by both methods from the aphid-inoculated plants. In the other 6 tests much better transmission was obtained from insect-inoculated plants than from mechanically inoculated plants. Infection was obtained from the mechanically inoculated plants only in the case of those in which the virus had undergone an incubation of at least 33 days. The results suggest that increase of virus takes place much more slowly in mechanically inoculated plants than in insect-inoculated plants. The observation that frequently symptoms appear more slowly in mechanically inoculated plants than in aphid-inoculated plants strengthens this suggestion.

The difference in symptoms frequently observed in plants inoculated with virus by means of aphids and in plants inoculated mechanically is shown in



Photographs by J. A. Carlile

FIG. 1. *Vicia faba* leaves. A. From plant inoculated by means of pea aphids. B. From healthy plant. C. From plant inoculated mechanically. D. From plant infected with the strain of virus from New Jersey. E. From healthy plant.

figure 1, A and C. Leaves from 2 plants, inoculated at the same time, were photographed 16 days after inoculation.

Two of the diseased plants in this experiment that failed to give infection at the first test were retested at a later time. One mechanically inoculated plant that failed to give transmission when tested 17 days after inoculation was retested 52 days after inoculation. In the second test, no virus was recovered from the diseased plant by means of aphids, but 3 of 5 plants inoculated mechanically with juice extracted from the plant eventually developed mild symptoms of disease. Another mechanically inoculated plant that failed to yield virus when tested 22 days after inoculation was retested 62 days after inoculation. In the second test no virus was recovered by means of aphids, but, again, 3 of 5 plants inoculated mechanically slowly developed mild symptoms of disease, demonstrating that in some cases diseased plants that fail to yield virus in one test may yield virus at a later time.

The experiment was continued by using as a source of virus the diseased plants obtained by sub-inoculation. In some cases plants showing severe symptoms were selected for testing. In other cases plants showing faint symptoms were tested. The results presented in table 2 show that there is

TABLE 2.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

Test number	No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
		By insects	By rubbing	By insects	By rubbing
1	34	0[a]	2	0	0
2	30	5	5	0	3
3	29	5	5	0	1
4	26	0	0	0	0
5	22	0	5	0	0
5a[b]	24	5	5	5	5
5b[c]	27	0	0	0	0
6	31	5	5	5	1
7	32	5	5	5	3
8n[d]	32	0	1	5	5
9	31	5	5	0	0
10	31	5	3	5	5
	Total	35	41	25	23

[a] Five *Vicia faba* plants were used in each test.
[b] 5a—Source plants that showed rapid spread of symptoms were selected.
[c] 5b—Source plants that showed slow spread of symptoms were selected.
[d] 8n—Source plants that showed slow spread and faint symptoms were selected.

considerable variability in transmission from aphid- as well as from mechanically inoculated plants. This test was made in the late fall and early winter under conditions that were not considered entirely satisfactory for uniform spread of the virus in the plants.

Since the experiments indicated that the length of the incubation period of the virus in the plants might be a factor influencing transmissibility, tests

were conducted with diseased plants that had been infected for short periods of time. Aphid-inoculated and mechanically inoculated plants that had been inoculated at the same time were tested simultaneously in adjoining cages in a greenhouse. In the tests for very short incubation periods, 50 aphids were placed on each inoculated plant for 1 day. Recovery of the virus was then determined by placing the aphids on 5 fresh *Vicia faba* plants and by crushing the inoculated plants and rubbing the juice into 5 fresh plants. The results are shown in table 3. The virus was not recovered from any plant

TABLE 3.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
	By insects	By rubbing	By insects	By rubbing
21	5[a]	2	0	0
14	5	5	0	0
8	2[b]	2[b]	0	2[b]
6	5	4		
4	2	2		
3	0	2[b]		
2	0	0		

[a] Five *Vicia faba* plants were used in each test.
[b] Faint or mild symptoms of disease produced.

within less than 3 days after inoculation. Virus was recovered by mechanical means from aphid-inoculated plants 3 days after inoculation and by means of aphids 4 days after inoculation. In one case very faint symptoms of disease were produced in 2 plants inoculated by rubbing with virus from mechanically inoculated plants tested 8 days after inoculation. After 14 and 21 days, respectively, virus was recovered from insect-inoculated plants but not from mechanically inoculated plants. These experiments demonstrated that virus is recoverable from aphid-inoculated plants 3 days after inoculation and at any time thereafter. They also showed that sub-inoculations from mechanically inoculated plants usually failed if made within 21 days after inoculation.

Serial Passage of Pea Virus 1 by Mechanical Methods

Experiments were made for the purpose of determining whether or not it would be possible to pass pea virus 1 in series mechanically. In one test a *Vicia faba* plant, severely infected 31 days after mechanical inoculation, was crushed and the juice rubbed into 5 fresh *V. faba* plants. Two of the 5 plants of the second passage became infected. After 130 days, leaves from these 2 plants were crushed and the juice rubbed again into 5 fresh *V. faba* plants. Two plants of the third passage showed mild infection. After 31 days, leaves from these 2 plants were crushed and rubbed into 5 fresh plants. One plant of the fourth passage developed very faint symptoms. Leaves from this plant were crushed after 38 days and rubbed into 5 fresh plants, but no symptoms of disease were produced.

In another test, 50 *Vicia faba* plants were inoculated with juice expressed from aphid-inoculated plants. Thirty-six became diseased. After 43 days, leaves from these plants were crushed and the composite sample of mixed juice was rubbed into 50 fresh plants. Eighteen plants of the second passage became diseased. Leaves from 16 of these were crushed 47 days later and the mixed juice from these was rubbed into 100 fresh plants. No symptoms developed in any of the plants of the third passage. When it became evident that no plants of the third passage were diseased as a result of mechanical inoculation, a test was made to determine if the virus could be recovered from the diseased plants of the second passage by means of aphids. For this test 3 diseased plants of the second passage were selected 60 days after inoculation. Aphids were fed on the plants for 3 days and then 75 were transferred to each of 10 small plants and allowed to feed for 3 days. No symptoms developed in any of the plants. These experiments demonstrate the difficulty of passing pea virus 1 in series mechanically.

Transmissibility from Plants Inoculated by Means of Single Aphids and from Plants Inoculated by Colonies of Aphids

Having observed that transmission was more certain and that symptoms appeared more quickly in plants inoculated by colonies of aphids than in plants inoculated by single individuals, it seemed of interest to test the transmissibility from such plants. The aphids used were large nymphs and adults that had been on diseased *Vicia faba* plants for 2 days. Colonies consisting of 30 individuals were placed on each of 20 *V. faba* plants and single aphids placed on each of another set of 20 plants. All of the plants exposed to colonies became diseased. Symptoms appeared in 4 to 5 days on 18 of the plants and in 6 to 7 days on the other two. Ten of the 20 plants exposed to single insects showed symptoms within 6 to 8 days and 3 others within 14 days. After 25 days, 5 plants from each lot were tested for transmissibility by both aphid and mechanical inoculation. Transmission was obtained in every instance from plants previously inoculated by colonies of aphids. Similarly, transmission was obtained in every instance by means of aphids from plants inoculated by single insects, but from only 2 of the 5 plants tested by means of mechanical inoculation. This experiment failed to indicate a significant difference in transmissibility from plants inoculated by colonies and those inoculated by single insects.

Properties of the Virus

Having found that pea virus 1 could be transmitted from aphid-inoculated plants by the carborundum powder method in a high percentage of trials, a series of experiments was undertaken to determine the physical properties of the virus. For this purpose diseased plants were crushed 4 to 8 weeks after inoculation by colonies of aphids, and the plant extract, after treatment, was rubbed into the leaves of small *Vicia faba* plants dusted with carborundum powder No. 320.

Thermal Inactivation. Resistance to heat was determined by placing 2 cc. of undiluted extract from diseased *Vicia faba* plants in thin test tubes, heating in a water bath at the desired temperature for 10 minutes, and then testing for infectivity by mechanical inoculation to *V. faba* plants. The results of several tests are summarized in table 4. Infection was obtained from juice

TABLE 4.—*Infections obtained by inoculation with virus exposed to different temperatures for 10 minutes*

Temperature (degrees C.)	No. plants inoculated	No. plants infected
Control	40	28
52	10	5
54	10	2
56	10	1
58	10	2
60	20	2
62	10	1
64	20	1
66	20	0

heated to various temperatures up to 64° C., but was not obtained from juice heated to 66° C.

Resistance to Aging in vitro. To determine resistance to aging *in vitro,* test tubes, each containing 2 cc. of expressed juice from diseased plants, were held at room temperature. After different intervals of time juice from the tubes was used for inoculation of *Vicia faba* plants. The results of these tests are shown in table 5. The virus was infective after aging *in vitro* for periods

TABLE 5.—*Infections obtained by inoculation with virus aged in vitro for different periods of time*

Time aged	Plants inoculated	Plants infected
2–6 hours	25	14
1 day	5	2
2 days	5	3
3 days	5	1
4 days	15	5[a]
5 days	15	0
7 days	5	0

[a] Symptoms slow to appear and of a mild type.

up to and including 4 days but was not infective after 5 days' aging. The appearance of symptoms in plants inoculated with virus that had aged for 4 days was delayed for a much longer period than in plants inoculated with juice that had aged for shorter periods.

Tolerance to Dilution. For determining tolerance to dilution, juice from diseased plants was diluted to the desired point with distilled water and then tested for infectivity by mechanical inoculation to *Vicia faba* plants. Since the thermal inactivation point and degree of resistance to aging *in vitro* found for pea virus 1 is very close to that previously shown for pea virus 2 (4), it seemed of interest to compare the tolerance to dilution by these viruses under

comparable conditions. The results are shown in table 6. Pea virus 1 was infective at a dilution of 10^{-3}, but was not infective at a dilution of 10^{-4}. Pea

TABLE 6.—*Infections obtained by inoculation with virus after various degrees of dilution*

Dilution	Pea virus 1		Pea virus 2	
	No. plants inoculated	No. plants infected	No. plants inoculated	No. plants infected
10^{-0}	50	36	20	18
10^{-1}	50	19	20	17
10^{-2}	50	12	20	17
10^{-3}	50	2	20	12
10^{-4}	50	0	20	4
10^{-5}	50	0	20	1

virus 2 was infective at a dilution of 10^{-5}. Dilutions greater than 10^{-5} were not tried.

Effect of Temperature on the Virus in the Aphids

Kunkel (2) has shown that the vector, *Macrosteles divisus* Uhl. (*Cicadula sexnotata* Fall.), always lost the ability to transmit aster yellows when held at a temperature of 32° C. for 1 day. The ability was regained on return to 24° C. Exposure for 12 days or more to a temperature of 32° C. permanently inactivated the virus without affecting the leaf hoppers. Since pea virus 1 is presumed to be held in the aphid vector in a manner similar to that in which aster-yellows virus is held in the leaf hopper, it seemed of interest to determine the effect of heat on pea virus 1 in the pea aphid.

In one experiment pea aphids were fed for 2 days on diseased *Vicia faba* plants in a cage in a greenhouse held at a temperature of 23° to 26° C. Seventy-five were then placed on each of 6 small *V. faba* plants in lamp chimneys in a room held at a constant temperature of 35° C. An equal number were placed on each of 6 small plants in the greenhouse. Both lots were transferred to fresh plants every day for 12 days. One lot was held in the hot room for 6 days and then in the greenhouse for 6 days, while the other lot was held in the greenhouse for the entire period. Of the 144 plants exposed, all became infected, demonstrating that the virus was still active in aphids that were held for 6 days at a temperature of 35° C.

In another test aphids that had fed on diseased plants were still infective after being held for 6 days at 35° C. on insusceptible red-clover plants.

In other experiments, requiring longer periods at 35° C., some of the colonies lost the ability to infect in 5 to 9 days and none were infective after periods longer than 9 days in the hot room. This loss of infectivity, however, may have been due to loss of infective individuals in the colonies. Mortality was higher than in the control colonies, and, after about 3 days in the hot room, the aphids no longer gave birth to normal nymphs. In one experiment the aphids appeared weak and were greatly reduced in number at the end of 8 days in the hot room. After 11 days, the colonies were reduced from

75 to an average of 5 individuals, whereas there was an average of 40 in the control colonies.

HOSTS

Broad bean (*Vicia faba*), garden pea (*Pisum sativum* L.), field pea (*P. sativum* var. *arvense* Poir.), sweet pea (*Lathyrus odoratus* L.) and crimson clover (*Trifolium incarnatum* L.) were reported in a previous paper to be susceptible to pea virus 1 (3).

Attempts to infect the following plants either by mechanical inoculation or by means of pea or potato aphids failed: red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), white dutch clover (*T. repens* L.), white sweet clover (*Melilotus alba* Desr.), yellow sweet clover (*M. officinalis* (L.) Lam.) and alfalfa (*Medicago sativa* L.). Attempts to infect peanut (*Arachis hypogaea* L.) with the pea aphid, and tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.) with the potato aphid were likewise unsuccessful. Sub-inoculations were made from each of these plants to *Vicia faba,* but in no case was the virus recovered. The Green Stringless Refugee and Corbett Refugee varieties of the garden bean (*Phaseolus vulgaris* L.) also failed to become infected when inoculated by means of the pea aphid. No infection was produced by mechanical inoculation to any of the following plants: Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L., *N. sylvestris* Spegaz. and Comes, *N. langsdorffii* Weinm., Green Stringless Refugee, Corbett Refugee and Robust varieties of the garden bean and mung bean (*P. aureus* Roxb.).

STRAINS OF PEA VIRUS 1

The virus used in the experiments reported here was the same strain of pea virus 1 that was employed in studies on transmission by the pea aphid (3). It was obtained originally from Westchester County, New York, and had been maintained in a greenhouse for a period of 6 years by serial passage in *Vicia faba,* using the pea or potato aphids as vectors. Another strain of pea virus 1 was obtained originally from pea aphids collected on pea plants growing near Princeton, New Jersey, and has been maintained in a similar manner for a period of 4 years by serial passage in *V. faba.*

The strain of virus from New York has already been described (3). The strain from New Jersey is essentially similar to the strain from New York in its transmission by aphids and in host range. It also produces the characteristic enations on the under surface of the leaves of crimson clover and peas and sometimes produces inconspicuous enations on the under surface of *Vicia faba* leaves. It differs slightly, however, in symptoms produced on *V. faba* and crimson clover and also in ease of mechanical inoculation. The strain from New York often produces large yellow spots on leaves of infected *V. faba* plants during early stages of invasion by the virus and sometimes these symptoms appear in the later stages as well. Figure 1, A and C, shows the large yellow spots frequently produced in *V. faba* plants diseased with the strain of virus from New York. In the first stages of invasion of young

V. faba plants the strain of virus from New Jersey often produces crinkling, distortion, and necrotic spots and splotches on the leaves. In later stages of invasion it is often difficult to distinguish the symptoms produced by the two virus strains. Figure 1, D, illustrates the spotted type of symptoms produced by the New Jersey strain of the virus in mature plants. When inoculated into crimson clover by aphids, early symptoms of the strain from New York consist of yellowish spots on the leaves, while, with the strain of virus from New Jersey, there is a tendency for the spots to be necrotic. No distinct differences have been observed in the symptoms produced by the two strains in garden-pea plants.

The strain of virus obtained in New Jersey has proved to be more difficult to transmit by mechanical inoculation in *Vicia faba* than the strain from New York. In some early experiments only a few infections were obtained. In a recent experiment, 50 plants were mechanically inoculated with juice expressed from plants diseased with the strain of virus from New Jersey and another 50 plants with juice extracted from plants diseased with the strain of virus from New York. Nine plants inoculated with the strain from New Jersey became infected, while 36 of the plants inoculated with the strain from New York became infected. It was because of this difference in degree of infectivity that experiments to determine properties of the virus *in vitro* were carried on with the strain of virus originally obtained in New York.

DISCUSSION

Previous papers on pea virus 1 (3, 5) have dealt largely with transmission by means of the pea and potato aphids. This was due to the fact that the virus is difficult to transmit by ordinary mechanical methods. In this respect it is similar to other viruses that require an incubation period in an insect vector. It has been demonstrated that pea virus 1 is readily transmitted by the carborundum-powder method. The results obtained, however, are quite different from those obtained with pea virus 2 (4), which is easily transmitted by ordinary rubbing methods and by aphids without the necessity of an incubation period in the vector. No difference in transmissibility of pea virus 2 has been observed between plants inoculated by aphids and those inoculated mechanically, and this virus is readily maintained by mechanical inoculation in serial passage in *Vicia faba*. On the other hand, there is a distinct difference in transmissibility of pea virus 1 from plants inoculated by colonies of aphids and from those inoculated mechanically. Sub-inoculations from mechanically inoculated plants have been more difficult to obtain and the virus is, therefore, not readily maintained in serial passage by mechanical transmission.

In plants inoculated mechanically the symptoms usually are milder and appear later than in plants inoculated by aphids. Apparently the virus spreads more slowly. These are symptoms often associated with "attenuation" of a virus. Whether or not the difference in transmissibility is due to

an "attenuation" of the virus is hard to say, since it is difficult to maintain the virus from these plants by serial passage in *Vicia faba.*

It is suggested that the difference in transmissibility may be attributable to the relative concentration of the virus in the plants. The virus may be present in a plant in sufficient concentration to produce symptoms and still not be present in sufficient concentration for transmission either by aphids or mechanically. It is possible that inoculations made by aphids fed on thoroughly diseased plants result in the virus reaching cells or tissues in which it multiplies and from which it spreads rapidly, whereas the virus inoculated by mechanical methods may often be localized in cells or tissues from which it spreads slowly. Occasionally, however, in a mechanically inoculated plant, symptoms appear promptly and are indistinguishable from those in the most thoroughly diseased plants inoculated by aphids. There seems to be no evidence, therefore, that the virus is changed by passage through the aphids.

It has been demonstrated that colonies of the pea aphid are more effective for the purpose of inoculating *Vicia faba* plants than is the carborundum-powder method. It should be emphasized, however, that it is necessary for the aphids to feed on thoroughly diseased *V. faba* plants in order to acquire the virus in sufficient amount for transmission to healthy plants. In fact, in some cases colonies of aphids have failed to acquire or transmit the virus from diseased plants from which it was recovered mechanically.

It has been difficult to transmit the virus from some aphid-inoculated plants. Whether this was because of the manner in which the plants were inoculated by aphids or whether it was because of the concentration of virus in the source plants from which it was obtained by the aphids is not known. The aphid-inoculated plants from which it has been difficult to transmit the virus have shown a mild type of symptoms. Plants inoculated by aphids after a short period of incubation of the virus in the aphids have sometimes produced very mild symptoms that were slow to develop. Similar results were sometimes obtained when inoculations were made by single insects. One test, however, failed to indicate a significant difference between transmissibility from plants inoculated by colonies of aphids and from plants inoculated by single aphids.

Snyder (8), Stubbs (9), Pierce (6), and Johnson and Jones (1) have reported on viruses in peas that produce the enations characteristic of pea virus 1. While these workers have not determined the mode of transmission by aphids, it seems quite evident that they were dealing with a virus similar to if not identical with pea virus 1. Stubbs suggested the name Enation Pea Mosaic for the disease and pea virus 1 for the virus producing it. Stubbs also pointed out that the Perfection variety of garden pea served to separate pea virus 1 from pea virus 2. The Perfection variety of pea is susceptible to pea virus 1 but is insusceptible to pea virus 2. The physical properties reported here for one strain of the virus agree fairly closely with those reported by Stubbs and Pierce. Johnson and Jones, however, appear to have been working with a strain more difficult to transmit by mechanical methods.

SUMMARY

Although pea virus 1 is difficult to transmit by the ordinary rubbing methods, it is transmissible by the carborundum-powder method.

Sub-inoculation from mechanically inoculated plants was found to be more difficult than from plants inoculated by colonies of aphids.

The virus was recovered from infected plants as soon as 3 days after inoculation by colonies of pea aphids. Sub-inoculation from mechanically inoculated plants usually failed if made within less than 24 days after inoculation, and in many plants after much longer periods of time. Sub-inoculation from aphid-inoculated plants failed in some cases.

The virus was carried through 4 serial passages by mechanical inoculation in *Vicia faba*. Sub-inoculation appeared to be even more difficult after several serial passages by mechanical inoculation in *V. faba*.

The virus was infective after heating *in vitro* for 10 minutes at various temperatures up to 64° C., but was not infective after heating to 66° C. It was infective after aging *in vitro* for periods up to 4 days, but was not infective after 5 days' aging. The virus was infective after dilution to 10^{-3}, but was not infective after dilution to 10^{-4}.

Aphids retained the virus for periods up to 8 days when they were removed from diseased plants and transferred to a succession of healthy plants that were held in a room at a temperature of 35° C. Failure of aphids to infect plants after being held for periods longer than 8 days at 35° C. may have been due to the loss of infective individuals in the colonies when held at this temperature.

Two strains of pea virus 1 are described. These differ in symptoms and in ease of transmission by mechanical means.

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A BACTERIAL BLIGHT OF STOCKS CAUSED BY PHYTOMONAS SYRINGAE

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In 1912 Briosi and Pavarino (2) reported on a disease of the flowering stock, *Matthiola incana* R. Br. var. *annua* Voss, and attributed the cause to a bacterium, which they named *Bacterium matthiolae,* n. sp. In a foot note they stated that the work would be published in the *Atti dell' Istituto Botanico dell' Universita di Pavia.* This second article (3) which was practically identical with the first, evidently appeared the same year in pamphlet form, since references have been found to it bearing the date 1912. It did not appear, however, in a volume of the above periodical until 1918. E. F. Smith (8), in reviewing the two publications, gave practically a complete translation. Rudorf and Job (7) reported the same disease in Argentina in 1932. This latter article has not been seen by the writer, but an abstract of it in the Review of Applied Mycology does not indicate that these investigators had the pathogen in culture. In 1934 Adams and Pugsley (1) reported a bacterial disease of flowering stock in Australia, and, while they described the pathogen, they declined to give it a name.

Both the Italian and the Australian organisms are green fluorescent bacteria, and, as far as described, they vary from *Phytomonas syringae* (van Hall) Bergey *et al.* in one character each. The Briosi and Pavarino pathogen was described presumably as gram-positive. E. F. Smith, translating literally from the Italian, states that "it resists Gram completely." It is not likely, however, that a green fluorescent bacterium is gram-positive. A few have been described but they have never been checked and proved correct. Bergey considers that his genus *Pseudomonas*, which contains the green fluorescent species, is a gram-negative genus. It is very easy to make a mistake in staining. The Adams and Pugsley pathogen is gram-negative, but it hydrolyzes starch. This might be correct, but the writer has worked with approximately 30 species of the green-fluorescent group, some of which have been reported to hydrolyze starch, and has found none that did. Maltose also was never utilized, although one would expect this if the starch were hydrolyzed. When the starch-agar-iodine method is used one should be careful to remove the colonies to be tested from the surface of a stiff agar. Otherwise, the iodine may not penetrate under the colonies and a light, spurious zone remains on the plate.

Since both these descriptions correspond so closely to that of *Phytomonas syringae*, the writer undertook to determine whether *Phyt. syringae* produces a disease of stocks and, if so, whether it resembles the disease described by these authors. Two isolates of this organism were on hand, one isolated from lilacs during the summer of 1937 and one from Lima beans isolated in November, 1937. Both were virulent cultures. When these isolates were inoculated

into flowering stock in the greenhouse a disease was produced similar to the ones described by Briosi and Pavarino and by Adams and Pugsley. The colored plates of Briosi and Pavarino assist in arriving at such a conclusion. Both strains of the pathogen were reisolated from the diseased tissues they produced. At the same time stocks also were inoculated with *Phyt. maculicola* (McCulloch) Bergey *et al.*, a bacterium similar in appearance and in biochemical reactions to *Phyt. syringae.* This pathogen was isolated July, 1937, from cauliflower, where it was causing a peppery spot of the leaves. No infection was obtained on stocks with the organism, although the cross inoculation was from one crucifer to another. The culture was virulent, since it produced infection both on cauliflower and on brussels sprouts at that time.

It is well known that *Phytomonas syringae* has a wide host range, but probably a definite one. That the stock is a host, we are certain from these experiments. The description of *Phyt. matthiolae* is so similar to *Phyt. syringae* that the two might be identical. The diseases they produce are similar. The writer, therefore, believes that *Phyt. matthiolae* is a synonym of *Phyt. syringae.*

The above bacterial disease of stock should not be confused with that reported by von Faber (5) and by Cooley (4) and attributed by them to *Phytomonas campestris* (Pammel) Bergey *et al.* Recently, this latter disease has been shown by Kendrick (6) to be caused by a pathogen similar in appearances to *Phyt. campestris,* but distinct. As yet, he has not named the organism.

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The Spread of Apple Mosaic.—In 1923 and 1924 it was reported[1, 2] that mosaic or variegation of apple, *Pyrus malus,* is transmissible by budding or grafting. Bradford and Joley[3] confirmed this finding, adding many observations on varietal behavior, and, further, uncovered the fact that this mosaic and its transmission by budding was reported in European literature early in the 19th century.

Following the transmission of this mosaic by budding and grafting in 1923, the author made various unsuccessful attempts to transmit the disease with various insects, mainly aphids and leaf hoppers. In the meantime many reports[4] had been received of its occurrence in various counties of New York State as well as from other States.

Since the preliminary attempts at transmission by insects had not given positive results, it seemed desirable to determine by survey methods whether there is any measurable natural spread. With this aim in view, a survey was made in 1927 of some of the orchards affected, followed by resurveys of the same orchards in 1932 and in 1937. Each tree in the orchards in question was examined and maps were prepared showing which trees were affected at each of these dates.

Many of these orchards are old and have suffered severely from winter injury, so that it is doubtful how many will last for another 5-year period. Also, the results to date are fairly conclusive, so that a brief report now seems desirable.

In the nine orchards surveyed in 1927, still extant in 1932, a total of 1207 trees was examined, of which 72 showed symptoms of mosaic in 1927 and 109 in 1932, thus making a 51.4 per cent increase for the 5-year period. At the end of the second 5-year period, a total of 981 trees was examined of which 96 showed mosaic in 1932, while at the end of the period, in 1937, there were 163 mosaic, an increase of 69.8 per cent. Since 3 orchards were removed and 1 added in the second period, not all these records extend the 10-year period; but, of 914 trees examined in both 1927 and 1937, there were 31 showing mosaic at the beginning and 138 at the end of the 10-year period. When examined by the X^2 test, all of these differences appear significant with odds higher than 100 to 1, the figures for the 10-year period being most highly significant.

In general, trees that once became affected remained so throughout

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[4] The first report and specimens were received from George Smith, special fruit disease agent in Orleans County, who believed spread was occurring in orchards under his observation. Numerous reports also were received from A. B. Burrell and W. D. Mills and several county agents.

this period, although there were very few foubtful cases that might have been due to errors in diagnosis or in mapping.

There are some indications, however, that this rather slow spread, should not be regarded as natural, *i.e.*, brought about through the agency of some insect vector, quite aside from the slowness of the spread. In several orchards, the trees recorded as mosaic at the time of the first observation, were largely in a single row, and subsequent spread had a tendency to follow the rows and in at least 2 cases in the direction followed in pruning operations.

Thus it appears certain that there has been a slow spread of the mosaic or variegation, but it appears probable that an explanation of this will be found connected with pruning operations rather than with insect transmission.—F. M. BLODGETT, Cornell University, Ithaca, N. Y.

An Apple Leafspot Associated with Fabraea maculata.—The Fabraea leaf blight of pear and quince is a common disease and is well known under a wide variety of environmental conditions in most parts of the world where these fruits are grown.[1] The writers are not aware, however, of any publication that describes the same organisms as causing a similar disease of apple. Massee,[2] in his general text on plant diseases, describes the organism as causing a leaf scald of "pear, apple, peach, quince, cherry, and other rosaceous fruit trees." Massee quotes the publications of Léveillé,[3] Sorauer,[4] and Galloway[5] as verification of his statement. In none of these is the disease described on apple, peach, or cherry.

During June, 1938, a severe case of leaf spotting of pear seedlings, French crabapple seedlings, and McIntosh apple scions, budded on various rootstocks, was observed in nursery rows at the U. S. Horticultural Station near Beltsville, Maryland. These observations led to the discovery that a great many of the lesions on the pear and the French crab leaves, and fewer on the McIntosh apple leaves, were bearing black, blisterlike acervuli typical of those caused by *Fabraea maculata* (Lév.) Atk. Microscopic examination revealed the presence of the typical *Entomosporium* conidia on the lesions on all the hosts. We have collected abundant specimens of the disease on the apple varieties and are prepared to distribute samples to investigators on request. —M. C. GOLDSWORTHY and M. A. SMITH, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

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3 Léveillé, J. H. Description d'un nouveau genre de champignons (*Entomosporium*). Bull. Soc. Bot. France 3: 30–32. 1856.

4 Sorauer, P. Fleckenkrankheiten oder Blattbraune der Birnen. Handb. der Pflanzenkrankheiten [2nd ed.] 2: 372–377. 1886.

5 Galloway, B. T. Leaf blight and cracking of the pear (*Entomosporium maculatum* Lév.). U. S. Dept. Agr. Rept. 1888: 357–384.

PHYTOPATHOLOGICAL NOTES

Danger in Unguarded Seed Importation.—World agriculture seems as yet hardly to comprehend the extent to which the seeds of plants serve as potential carriers of destructive plant pathogens prevalent in different regions of the earth. In spite of Orton's excellent bulletin on seed-borne parasites[1] and other efforts to place before the public the growing evidence of the seed-dissemination of a wide range of plant diseases, there still appears to be very little public recognition of the fact that unguarded transportation of seeds from one continent to another is fraught with the danger of introducing previously absent disease-producing agents or biotypes into new regions. Moreover, there seems to be but slight general appreciation of the fact that the long and intensive work by plant breeders to build up superior varieties of crop plants resistant to the major diseases prevalent in their respective countries is constantly threatened with failure as long as there is danger of the seed-introduction of new parasites to which such superior varieties may be susceptible.

The importance of these considerations is emphasized by such announcements as that in July, 1938,[2] of a new anthracnose (*Colletotrichum* sp.) of cotton found in Manchukuo. No one knows the possible damage that might result were this introduced into America, but when it is considered that the ordinary cotton anthracnose (*C. gossypii*), seed-carried, was the predominant cause of destructive seedling blight in 9 important cotton States in 1938,[3] it at once becomes evident that a new cotton anthracnose accidentally introduced in seed might well have serious consequences like those confronting American mint growers from the spread of mint anthracnose[4] or American potato growers from the spread of the destructive bacterial tuber ring rot and wilt[5] both of which perhaps have been introduced unwittingly in propagating stock from some other continent.

It is a matter of satisfaction that a subcommittee of The American Phytopathological Society,[6] headed by M. T. Munn of the New York State Experiment Station, Geneva, is giving serious consideration to the problem of dealing more effectively with seed-borne plant diseases. This committee deserves active support in its constructive endeavors.—HOWARD P. BARSS, Washington, D. C.

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[6] Orton, C. R. Report of Committee on Regulatory Work and Foreign Plant Diseases. Phytopath. **28**: 298. 1938.